ASSOCIATION OF INTIMATE PARTNER VASECTOMY, USE OF LONG-ACTING PROGESTOGEN-BASED CONTRACEPTIVES AND INTRA-UTERINE CONTRACEPTIVE DEVICES WITH RISK OF OVARIAN CANCER

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17β-HSD	17-β-hydroxysteroid dehydrogenase
ALL	Adult literacy and life skills
ABP	Androgen binding protein
AMH	Anti-Müllerian hormone
AR	Androgen receptor
ARGs	Accessory reproductive glands
ARVs	Antiretroviral drugs
ASA	Acetylsalicylic acid
BMI	Body mass index
BOT	Benign ovarian tumour
BRCA	Breast cancer susceptibility gene
BSO	Bilateral salpingo-oophorectomy
BTL	Bilateral tubal ligation
CA-125	Cancer antigen 125
CHCs	Combined hormonal contraceptives
CI	Confidence interval
CICs	Cortical inclusion cysts
COCs	Combined oral contraceptives
COX	Cyclo-oxygenase
Cu-IUDs	Copper bearing intrauterine devices
DAO	Diamine oxidase
DHEA	Dihydroepiandrosterone
DHT	Dihydrotestosterone
DMPA	Depot medroxyprogesterone acetate
DNA	Deoxyribonucleic acid
DSS	Disease specific survival
DVT	Deep vein thrombosis
E_2	Oestradiol
EAOC	Endometriosis associated ovarian cancer
EOC	Epithelial ovarian cancer
EPT	Oestrogen-progestin preparations

ER	Oestrogen receptor
ERα	Oestrogen receptor alpha
ERβ	Oestrogen receptor beta
ET	Oestrogen-only therapy
FIGO	International Federation of Gynecology and Obstetrics
FSH	Follicle stimulating hormone
FSHR	Follicle stimulating hormone receptor
FTSC	Fallopian tube serous carcinoma
GCTs	Germ cell tumours
GICs	Germinal inclusion cysts
GnRH	Gonadotropin releasing hormone
GOG	Gynecology oncology group
hCG	Human chorionic gonadotropin
HDLs	High density lipoproteins
HIV/AIDS	Human immunodeficiency virus/Acquired immune deficiency syndrome
HGSC	High-grade serous carcinoma
HGSOC	High-grade serous ovarian cancer
HNPCC	Hereditary nonpolyposis colorectal cancer
HOSE	Human ovarian surface epithelia
HPV	Human papillomavirus
HRT	Hormone replacement therapy
HR	Hazard risk
HWY	Hundred woman years
IGF	Insulin growth factor
IGFBPs	IGF binding proteins
IRR	Incidence rate ratio
IUDs	Intrauterine contraceptive devices
LARCs	Long-acting reversible contraceptives
LDLs	Low density lipoproteins
LH	Luteinizing hormone
LHR	Luteinizing hormone receptor
LGSC	Low-grade serous carcinoma
LMP	Low malignant potential

LNG/ETG	Levonorgesterel/etonorgesterel
LNG-IUS	Levonorgesterel releasing intrauterine system
LUF	Luteinised unruptured follicle
MELAA	Middle Eastern/Latin American/African
MI	Myocardial infarction
MMPs	Matrix metalloproteinases
MMS	Multimodal screening
MPA	Medroxyprogesterone acetate
NET-EN	Norethisterone enanthate
NHS	Nurses' Health Study
NK	Natural killer
NPV	Negative predictive value
NSAIDS	Non-steroidal anti-inflammatory drugs
NZCR	New Zealand Cancer Registry
OCs	Oral contraceptives
OR	Odds ratio
OS	Overall survival
OSE	Ovarian surface epithelia
PAP	Prostatic acid phosphatase
PAO	Polyamine oxidase
PBSO	Prophylactic bilateral salpingo-oophorectomy
PCOS	Polycystic ovary syndrome
PE	Pulmonary embolism
PFS	Progression free survival
PGE	Prostaglandin E
PGF	Prostaglandin F
PID	Pelvic inflammatory disease
PMH	Post-menopausal hormone
POICs	Progestogen-only injectable contraceptives
POPs	Progestogen-only pills
PPV	Positive predictive value
PR	Progesterone receptor
PRA	Progesterone receptor A

PRB	Progesterone receptor B
RCT	Randomised controlled trial
RMI	Risk of malignancy index
RNA	Ribonucleic acid
ROCA	Risk of Ovarian Cancer Algorithm
ROS	Reactive oxygen species
RR	Relative risk
RRSO	Risk reducing salpingo-oophorectomy
SCSTs	Sex cord-stromal tumours
SEE-FIM	Sectioning and extensively examining the fimbriae
SHBG	Sex hormone-binding globulin
SIR	Standardized incidence ratio
SLE	Systemic lupus erythematosus
SMR	Standardised mortality ratio
SPSS	Statistical package for the social sciences
STDs	Sexually transmitted diseases
STICs	Serous tubal intraepithelial carcinomas
T	Testosterone
TB	Tuberculosis
TEC	Tubal epithelial cells
THBSO	Total hysterectomy and bilateral salpingo-oophorectomy
TL	Tubal ligation
TVS	Transvaginal ultrasound
USS	Ultrasound screening
VEGF	Vascular endothelial growth factor
VLDLs	Very low density lipoproteins
VTE	Venous thromboembolism
WHO	World Health Organization

ABSTRACT

Background: Ovarian cancer is an aggressive and fatal disease affecting women worldwide. Though the pathogenesis and causes of ovarian cancer have not been established, some risk factors are well known. Use of oral contraceptives and tubal ligation have been shown to be inversely associated with ovarian cancer. What has not been clearly demonstrated is the association between intimate partner vasectomy, use of intrauterine contraceptive devices, and long-acting progestogen-based contraceptives and the risk of ovarian cancer. The limited scope for early detection of this neoplasm, and poor survival despite advances in treatment, make prevention especially important.

Objectives: This study sought to investigate whether there were associations between the use of long-acting progestogen-based contraceptives, intrauterine contraceptive devices (IUDs), and vasectomy of a woman's sexual partner, and ovarian cancer.

Methods: This was a New Zealand nationwide population-based case-control study involving women aged 35-69 years. Controls were randomly selected from the New Zealand electoral roll. Cases were women with a diagnosis of incident ovarian cancer recruited from the New Zealand Cancer Registry (NZCR) and had to be listed on the electoral roll. A postal questionnaire was used to gather information on socio-demographic characteristics, contraceptive use, and risk factors for ovarian cancer. Data were analysed using IBM Statistical Package for the Social Sciences (IBM SPSS statistics 22). Age-adjusted analyses were done using the method of Mantel and Haenszel. In multivariate analyses, binary logistic regression was used. Approval to conduct the study was obtained from the Southern Health and Disability Ethics Committee (13/STH/26) and the University of Canterbury Human Ethics Committee (HEC 2013/08).

Results: Between 1st May 2013 and 31st October, 2015, 1,903 controls were approached. Of these 1,735 were eligible for the study and 837 participated in the study (response proportion of 48.2%). Of the 837 participants, after reviewing the questionnaires, 91 were excluded because they had prior history of bilateral oophorectomy or ovarian cancer, or were more than 69 years of age. In the same period, 258 cases were received from NZCR out of whom 205 were eligible and 152 took part (response proportion of 74.1%). Ever-use of vasectomy was inversely associated with ovarian cancer (OR = 0.67; 95% CI = 0.46-0.96). Each year of use was associated with an OR of 0.97 (95% CI = 0.94-1.00). Ever-use of DMPA was associated with an OR of 0.70 (95% CI = 0.38-1.30). Age at first use, duration of use, and

time since last use of DMPA were not statistically significantly associated with ovarian cancer. Although ever-use of IUDs was not associated with ovarian cancer (OR = 0.98; 95% CI = 0.66-1.47), longer duration of use was associated with higher risk (P-trend = 0.030) and longer time since last use was inversely associated with ovarian cancer (P-trend = 0.032). There were also statistically significant inverse associations between ovarian cancer and use of oral contraceptives, parity, and breastfeeding.

Conclusion: The findings of this study suggest that ovarian cancer may be inversely associated with use of DMPA and partner vasectomy and positively associated with use of intrauterine contraceptive devices. However, these findings are not definitive; a study with greater power, or a collaborative analysis of existing studies, is needed to better assess these associations.

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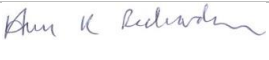
Name: *Ann Richardson* Signature:  Date: *24/05/2016*

CHAPTER 1: INTRODUCTION

This thesis presents a New Zealand nationwide population-based case-control study involving women aged 35 to 69 years. The study sought to investigate whether there were associations between the use of long-acting progestogen-based contraceptives and intrauterine contraceptive devices and ovarian cancer. It also aimed to find out whether vasectomy of a woman's sexual partner was associated with ovarian cancer.

Worldwide, over 225,000 women are diagnosed with, and over 140,000 women die from, ovarian cancer each year [1, 2]. Despite advances in treatment, ovarian cancer causes considerable morbidity, and long-term survival remains poor, with no available screening methods shown to decrease mortality; thus, primary prevention would be a major advance. In New Zealand, in 2012 ovarian cancer was the eighth most common cancer in women, accounting for 3% of cancer registrations, and the fifth most common cause of cancer death in women, accounting for 4% of female deaths from cancer [3]. In 2012 in New Zealand, 266 women were diagnosed with ovarian cancer and 175 women died from ovarian cancer [3]. During the period 1994 to 2007, New Zealand women with ovarian cancer had a five-year relative cumulative survival ratio of 0.426, and a 10-year relative cumulative survival ratio of 0.380 [4].

Previous studies have shown that use of oral contraceptives is inversely associated with ovarian cancer with studies consistently finding a 30-40% lower risk of ovarian cancer for ever-use compared to never-use [5-7]. The inverse association is more marked with longer duration of use, and is estimated at 20% for up to five years use, and nearly 60% for 15 years or more [6, 8]. Similarly lower risks have been found even among women with *BRCA* mutations, albeit from a higher baseline. A meta-analysis of oral contraceptive use and ovarian cancer in *BRCA1/2* carriers found a 50% lower risk for ever-users, with a 20% lower risk for each five years of use [9]. Worldwide, if the association is regarded as causal, oral contraceptives are estimated to have prevented over 200,000 women from developing ovarian cancer and more than 100,000 women dying from ovarian cancer [6].

Although a few studies have investigated the association between use of long-acting progestogen-based contraceptives and intrauterine contraceptive devices and ovarian cancer, findings have been inconsistent. The WHO Collaborative Study of Neoplasia and Steroid Contraceptives found no association between ever-use of depot medroxyprogesterone acetate (DMPA) and ovarian cancer, and showed no consistent patterns of higher or lower risk with

duration of use, time since first or most recent use, or age at first use of DMPA [10]. A study in Shanghai found a statistically non-significant higher risk of ovarian cancer with use of DMPA (odds ratio = 2.8; 95% CI = 0.9-8.5) and no statistically significant trend with years of use [11]. A recent case-control study carried out in South Africa found an odds ratio of 0.69 (95% CI = 0.36-0.99) for oral and injectable contraceptive use and an odds ratio of 0.35 (95% CI = 0.17-0.71) for exclusive use of injectable contraception [12]. A multicentre case-control study of DMPA and epithelial ovarian cancer in Thailand found an odds ratio of 0.61 (95% CI = 0.44-0.85) for ever-use and an odds ratio of 0.17 (95% CI = 0.07-0.39) for more than three years of use of DMPA [13].

Similarly, results from studies of use of intrauterine contraceptive devices (IUDs) and ovarian cancer have been inconsistent. Shu et al [11] found an odds ratio of 0.5 (95% CI = 0.21-1.1) for ever-use of IUDs. Ness et al [14], in a large case-control study, found an odds ratio of 0.75 (95% CI = 0.59-0.95) for ever-use of IUDs. In contrast, a case-control study nested in the Nurses Health Cohort Study, found an odds ratio of 1.76 (95% CI = 1.08-2.85) in IUD users [7].

Some research has been published on the effects of vasectomy on ovarian cancer, again with inconsistent results [7, 14, 15], with odds ratios of 0.77 (95% CI = 0.61-0.99) [14] and 0.87 (0.63-1.19) [7] in two case-control studies and a relative risk of 1.25 (0.85-1.84) in a prospective cohort study [15].

Although the association between use of these contraceptives and ovarian cancer is not clear, many women use these methods of contraception. DMPA, IUDs, and vasectomy have been, and continue to be, in widespread use in New Zealand and it is useful to investigate their association with ovarian cancer because:

- Not all women can take combined oral contraceptives because of unwanted effects or contraindications such as a history of venous thromboembolism, a known germline thrombogenic mutation, migraine with aura, cardiovascular disease, hypertension, smoking in women over the age of 35 years, uncontrolled diabetes, systemic lupus erythematosus (SLE), or active liver disease [16, 17].
- Improved knowledge of the association with a range of contraceptives may shed light on the pathogenesis and prevention of ovarian cancer.

It is important to assess the impact of these contraceptives on overall risk of ovarian cancer. Knowledge of the association between these contraceptives and ovarian cancer may have an impact on family-planning options, especially for women at high risk of ovarian cancer, and may help to increase the range of possibly effective preventive strategies. Ovarian cancer is an aggressive and fatal disease. The limited scope for early detection and cure of this neoplasm makes prevention especially important. Therefore, the following research questions were developed.

1.1 RESEARCH QUESTIONS

1 What is the association between use of long-acting progestogen-based contraceptives, and intrauterine contraceptive devices, and ovarian cancer?

2 Is vasectomy of a woman's sexual partner associated with ovarian cancer?

1.2 HYPOTHESES

1. Use of long-acting progestogen-based contraceptives is inversely associated with ovarian cancer.
2. Use of intra-uterine contraceptive devices has a positive association with ovarian cancer.
3. Vasectomy in a woman's sexual partner is inversely associated with ovarian cancer.

1.3 OBJECTIVES

1. To establish whether there is an inverse association between ovarian cancer and use of progestogen-based long-acting contraceptives.
2. To quantify the possible variation in risk of ovarian cancer (if any) associated with variation in duration of use of long-acting progestogen-based contraceptives.
3. To determine the association between intra-uterine contraceptive devices use and ovarian cancer.
4. To determine the association between partner's vasectomy and ovarian cancer.

1.4 THESIS ORGANISATION

The thesis begins with a review of the literature on ovarian cancer and contraceptives. Chapter 2 covers classification, grading, and staging of ovarian cancer, proposed theories of pathogenesis of ovarian cancer, risk factors for ovarian cancer and their proposed biologic mechanisms of effect, and prognostic indicators for ovarian cancer. Chapter 3 provides an overview of available contraceptives including their modes of action. In Chapter 4, issues regarding the association between contraceptive use and ovarian cancer are discussed. This

includes a summary of epidemiologic studies assessing the association between use of contraceptives (DMPA, IUDs, and partner vasectomy) and ovarian cancer, proposed biologic mechanisms of effect, and an overview of the association between contraceptive use and cancer.

Chapter 5 discusses the methods used in carrying out this study. The chapter includes a description of the participants, including the age and geographic location of all women approached and those who agreed to participate. In this chapter, the stage and histological types of ovarian cancer in respondents and non-respondents among cases are compared.

The findings of the study are presented in Chapters 6 to 10. The prevalence of contraceptive use in New Zealand women aged 35 years and above (estimated from the population-based controls in the case-control study), and a comparison with a previous population-based study carried out by Paul et al. [18] are presented in Chapter 6. In Chapter 7, the results of the analysis of factors known to be associated with lower or higher risk of ovarian cancer are presented. Findings of this study on the association between use of DMPA, IUDs, and partner vasectomy, and risk of ovarian cancer are presented in Chapters 8, 9, and 10. In discussing the findings of this study (Chapters 6-10) comparisons with findings of previous studies are made. Chapter 11 presents a summary of the findings, a discussion of strengths and limitations of the study, implications of the findings, conclusions, and recommendations for future research.

1.5 COLLABORATORS

This research was done in collaboration with the following people: Dr Mary Jane Sneyd, Mrs Pat Coope, Professor Ann Richardson and Professor John Potter assisted in the development of the research protocol and in the application for funding. Mrs Pat Coope assisted in random selection of participants from the electoral roll and provided statistical advice throughout the study. We had one research assistant, Catriona Mackay, who worked part-time and was responsible for sending the questionnaires. Interview of participants who did not respond to two mail-outs was conducted by Catriona Mackay, myself, and Professor Ann Richardson. My supervisors reviewed drafts of the thesis and provided guidance throughout the study. I formulated the research question and was involved in the entire study process. I did statistical analysis under the guidance of Mrs Pat Coope (a statistician).

CHAPTER 2: OVARIAN CANCER

In this chapter, classification, grading, staging and pathogenesis of ovarian cancer are discussed. In addition, risk and inversely related factors (here, for ease of understanding, referred to as protective factors without any implication that these are known to be causal) for ovarian cancer previously reported in other studies are presented, and their mechanism of effect on the pathogenesis of ovarian cancer discussed. The chapter ends with discussion of currently available screening methods and preventive strategies and prognostic indicators in relation to survival from ovarian cancer.

2.1 CLASSIFICATION, STAGING, AND GRADING

The ovaries are almond-shaped organs located on either side of the uterus within the pelvis [19]. The ovary is in contact with the fimbrial end of the fallopian tube which then connects it with the rest of the female genital tract (uterus, cervix, and vagina) [20]. Structurally, the ovary is organised into: the cortex, which contains follicles; an inner medulla, which mainly consists of stroma and steroid-producing cells; and the hilum, which is the entry point of nerves and blood vessels and contains predominantly hilus cells (identical to Leydig cells in the testis). A single layer of cells covers the outer surface of the ovary (ovarian surface epithelium). Follicles are composed of an oocyte surrounded by hormone-producing granulosa and theca cells [19, 21]. The ovaries have 2 functions; production of germ cells and hormones [21].

The ovaries (and testis) develop from three sources: the ovarian surface epithelium is derived from the coelomic epithelium, the stroma from the mesenchyme, and the oocytes from primordial germ cells (from the yolk sac) [19, 22]. In line with this, ovarian cancers are divided into 3 histopathologic categories: epithelial, germ cell, and sex cord and stromal - according to the presumed cell of origin [19, 23, 24]. Tumours from other sites (mainly breast, gastrointestinal tract, and reproductive tract tumours) may also metastasize to the ovary and account for 5-6% of all ovarian tumours [23].

The majority of ovarian tumours are epithelial (60% of all ovarian tumours and 90% of malignant ovarian tumours). Epithelial tumours are further classified into 5 main histological subtypes: serous, mucinous, endometrioid, clear cell, and transitional (Brenner) [19, 23, 24]. Other histological subtypes include: mixed epithelial tumours which consist of more than one subtype [19, 24, 25]; undifferentiated (no distinct microscopic features) [23, 24]; and adenocarcinoma not otherwise specified (adenocarcinoma NOS), which are malignant

epithelial ovarian tumours not classified into any specific subtype [19, 26]. Most epithelial ovarian cancers resemble epithelial cells found in other sites: serous tumours resemble the fallopian tube epithelium; mucinous tumours resemble endocervical or intestinal epithelia; endometrioid tumours resemble the endometrial lining; and transitional cell tumours resemble the urothelium [19, 24-26].

Epithelial ovarian cancer (EOC) mainly occurs in postmenopausal women with a peak in the 6th decade [23]. Depending on the clinical behaviour and pathologic characteristics of the tumour, all histologic subtypes of epithelial ovarian cancer can be classified into borderline (also known as tumours of low malignant potential [LMP]) and malignant (invasive) tumours [19, 24, 25, 27]. Borderline ovarian tumours (BOT) account for about 10% of all epithelial ovarian tumours [25]. They are mainly mucinous or serous, and are capable of metastasis. Compared to the invasive EOC, they are usually diagnosed in younger women, are detected at an earlier stage (75% in stage 1), and have a better prognosis [25, 28].

Germ-cell tumours (GCTs) account for 25% of all ovarian tumours and 3-7% of invasive ovarian cancer [19]. They include dysgerminomas, endodermal sinus tumour (yolk sac tumours), embryonal carcinoma, polyembryoma, choriocarcinoma, and teratoma. Mixed subtypes also occur. They are mainly diagnosed in women in their 20s and 30s and, compared to the epithelial ovarian tumours, have a better prognosis. Most of these tumours produce biologic markers (human chorionic gonadotropins and alpha-fetoprotein), which can be used in detection and monitoring of treatment of the tumour [19, 23]. The main prognostic determinant of germ-cell tumours is the stage at diagnosis. GCTs are not usually graded other than as immature teratomas in which the extent of immature tissue is used to determine grade [19].

Sex-cord-stromal tumours (SCSTs) are thought to arise from cells that are derived from the mesenchyme [24]. They include granulosa-cell tumours, thecomas, fibromas, and sertoli-Leydig-cell tumours. Most of these tumours produce hormones (oestrogens and androgens) which may cause virilisation, precocious puberty, or post-menopausal bleeding [19, 23]. They account for 5-8% of all ovarian tumours [19, 23] and 7% of invasive ovarian cancers [19]. Most are diagnosed at an early stage (stage 1) and, as such, are usually amenable to surgery and the prognosis is good [19]. SCSTs are diagnosed across all age groups but mainly occur in women in their 4th and 5th decades [29]. Other non-epithelial tumours are carcinosarcoma and small cell tumors [25].

In line with recent theories regarding pathogenesis, epithelial ovarian cancer can also be classified as type I and II, or type I, II, and III tumours, based on cell of origin and type of mutations [30, 31]. Type I tumours include low-grade serous carcinoma (LGSC), low grade endometrioid tumours, clear-cell carcinoma, and mucinous tumours, whereas type II tumours include high-grade serous carcinomas (HGSC), high-grade endometrioid tumours, carcinosarcomas and undifferentiated carcinomas [30-32]. Type I tumours account for 25% of all EOCs while type II account for the other 75% [32]. Ovarian tumours can also be classified as type I (low grade serous and mucinous tumours), type II (endometrioid and clear-cell tumours), and type III (high-grade serous and undifferentiated tumours) [30, 31]. Type III tumours have the worst prognosis [31]. Classifying into type I/II or type I/II/III in epidemiologic studies may be difficult because this requires extensive sectioning and molecular profiling during histopathological examination [31].

2.1.1 STAGING

Staging of ovarian cancer is achieved by surgical and radiologic evaluation at initial assessment [19, 23, 24]. However, some patients undergo adjuvant chemotherapy followed by surgery, at which time staging is done [25]. For comprehensive surgical staging of ovarian cancer, hysterectomy, bilateral salpingo-ooporectomy, and omentectomy are required. In addition, complete exploration of the abdomino-pelvic cavity for metastatic tumours, sampling of pelvic and para-aortic lymph nodes, and ascites fluid or peritoneal washing are required [23, 25].

The International Federation of Gynecology and Obstetrics (FIGO) categorises ovarian cancer into 4 stages (Table 2.1) [19, 23, 24, 33]. The stages are determined by the extent of disease at the time of diagnosis, and are designated I to IV [33].

Table 2.1: International Federation of Gynecology and Obstetrics (FIGO) staging of ovarian cancer

Stage I:	Tumour limited to the ovaries	
	IA:	Tumour limited to one ovary.
	IB:	Tumour limited to both ovaries.
	IC:	Tumour involves one or both ovaries plus: capsule ruptured, tumour on ovarian surface or presence of malignant cells in ascites or peritoneal washings.
Stage II:	Tumour involving one or both ovaries with pelvic extension	
	IIA:	Extension to the uterus and/or the fallopian tube
	IIB:	Extension to other pelvic tissues
	IIC:	Extension to the uterus, fallopian tubes or other pelvic tissues plus: capsule ruptured, tumour on ovarian surface or presence of malignant cells in ascites or peritoneal washings
Stage III:	Tumour extension to the abdominal cavity	
	IIIA:	Microscopic abdominal peritoneal metastases
	IIIB:	Macroscopic peritoneal metastases beyond the pelvis (≤ 2 cm in size)
	IIIC:	Macroscopic peritoneal metastases beyond the pelvis (> 2 cm in size) and/or involvement of the pelvic, paraaortic, or inguinal lymph nodes
Stage IV:	Distant metastases: pleural effusion positive for malignant cells; pulmonary, liver, or splenic parenchymal metastasis; involvement of the supraclavicular lymph nodes or skin.	

Most ovarian tumours (75%) are diagnosed at an advanced stage (stage III and IV), 20% are diagnosed in stage I, and 5% in stage II. Lack of an anatomic boundary between the pelvic and abdominal cavities has been proposed as the reason for the small proportion of patients diagnosed with stage II disease. Stage is a major prognostic factor for ovarian cancer survival. The 10-year survival proportion for stage I is 73%; stage II, 45%; stage III 21%; and stage IV <5% [25]. Serous tumours are usually diagnosed at an advanced stage (stage III or IV), whereas clear-cell, endometrioid, and mucinous tumours are usually in stage I at the time of diagnosis [24].

2.1.2 GRADING

Microscopic examination is necessary for confirmation of diagnosis, histologic typing and grading of ovarian cancer [19, 23]. However, there is lack of universal agreement on a standard grading system for ovarian cancer [34, 35]. The grading systems differ by histopathologic features used to determine grade [35] and have poor reproducibility [34]. The grading systems mainly used are the World Health Organization (WHO), the International Federation of Gynecology and Obstetrics (FIGO), and mainly in the USA, the Gynecology Oncology Group (GOG) [35].

2.2 DIAGNOSIS

Symptoms commonly experienced by women with ovarian cancer include abdominal bloating, pelvic or abdominal pain, urinary urgency or frequency, and indigestion. Others

include the sensation of a pelvic mass, abdominal swelling, change in stool calibre, early satiety, and abnormal vaginal bleeding [23, 36]. Unfortunately, these symptoms are usually vague, non-specific, and frequent in women without ovarian cancer, and some are signs of advanced disease [23, 25, 36, 37].

Methods available for initial detection of ovarian cancer include pelvic examination, pelvic ultra sound, and measurement of serum levels of cancer antigen 125 (CA-125) [23, 25]. These tests have sensitivity and specificity too low to qualify for population screening [23, 25, 38-40]. Surgery is useful in the diagnosis, staging, and treatment of ovarian cancer [23, 25].

2.3 TREATMENT

Ovarian cancer is treated by surgical debulking followed by platinum-based chemotherapy. In some patients, surgical debulking is not possible at initial diagnosis, in which case neoadjuvant platinum-based chemotherapy is administered followed by surgery [23, 25]. There are ongoing trials of other treatment modalities including immunotherapy [41, 42] and hormone therapy [25]. CA-125 can be used in the monitoring of treatment [23, 25].

Ovarian cancer histological subtypes have different responses to chemotherapy [25, 43, 44]. High-grade serous tumours have a high sensitivity (80%) compared to clear-cell carcinoma (15%) [25, 44]. Mucinous tumours also have low sensitivity to platinum-based chemotherapy [43, 44]. It has been proposed that ovarian cancer histological subtypes should be managed as different disease entities [43, 44].

2.4 PATHOGENESIS OF OVARIAN CANCER

As discussed earlier, ovarian cancer is classified into three histopathologic groups - epithelial, germ-cell and sex cord-stromal - based on the cell type of origin [23]. Epithelial tumours are the most common, accounting for 90-95% of ovarian tumours [45]. They include serous, endometrioid, clear-cell, mucinous, transitional cell (Brenner), and undifferentiated ovarian tumours[23]. There is ongoing debate regarding ovarian cancer pathogenesis [46].

Ovarian cancer has traditionally been viewed as one disease entity that undergoes progressive change from well to moderate to poorly differentiated cells, followed by extension to the pelvis and abdomen, and finally distant metastases. Recent studies have led to the classification of epithelial ovarian cancer into type I and type II tumours based on shared mutations, histogenetic pathways, and behaviour [47-49]. Type I tumours include low-grade

serous, low-grade endometrioid, Brenner tumours, clear-cell, and mucinous carcinomas. They are generally low grade, slow growing, present at an early stage (stage I), are relatively stable genetically, and have a good prognosis. They are associated with mutations in *KRAS*, *BRAF*, *ERB2*, *PTEN*, *PIK3CA*, *ARIDIA*, and *CTNNB1/β-catenin*, and rarely have *p53* mutations [47, 48, 50, 51]. Type I tumours have been found adjacent to benign epithelial tumours [50], and are thought to develop sequentially from cortical inclusion cysts (CICs), to borderline tumours, and finally to invasive tumours [47, 48, 51]. Type II tumours are comprised mainly of high-grade serous ovarian cancer (HGSOC), others are high-grade endometrioid, malignant mixed mesodermal tumours (carcinosarcomas), and undifferentiated carcinomas [48]. They present in advanced stage (stage II-IV), grow rapidly, are genetically unstable, and show *p53* mutations. They rarely harbour mutations found in type I tumours and are not found with adjacent borderline tumours [47, 48].

Epithelial ovarian tumours have been thought to arise from the ovarian surface epithelium (OSE), which is of coelomic origin. In this tumourigenic pathway, the OSE invaginates forming CICs; then, under the influence of local factors (possibly steroidal hormones), it undergoes metaplasia followed by neoplastic transformation [52]. Theories have been put forward to explain this. Incessant-ovulation is thought to play a role in ovarian carcinogenesis (incessant-ovulation theory). Ovulation results in repeated disruption and repair of the epithelium, which provides an opportunity for gene mutation that may lead to the initiation of cancer [23, 50, 53]. Ovulation has also been viewed as an inflammatory process; in this case, the inflammatory mediators may cause DNA damage leading to neoplastic transformation [48, 50, 54]. Supporting a role for inflammation is the observed lower risk of ovarian cancer with use of non-steroidal anti-inflammatory drugs [55]. In addition, the release of follicular fluid during ovulation, which has been shown to contain reactive oxygen species, may play a role in ovarian carcinogenesis [48, 54]. In the incessant-ovulation theory, the greater the total number of life-time ovulatory cycles, the higher the risk. This is supported by the observed lower risk seen with parity, use of oral contraceptives, and breast feeding.

The OSE has receptors for gonadotrophin releasing hormone, follicle stimulating hormone, luteinising hormone, androgens, oestrogen, and progesterone [50]. The gonadrotrophin hypothesis proposes that exposure to a high concentration of the hormone leads to malignant transformation of the OSE. This can either be by the gonadotrophins stimulating the OSE directly, or through stimulation of increased production of oestrogen by the ovary, which, in turn, stimulates the OSE, enhancing transformation. This is supported by the higher risk seen

in conditions of high gonadotrophin levels such as polycystic ovary syndrome (PCOS) and menopause, and the inverse association with oral contraceptive use and parity [50, 53, 55]. Pregnancy and use of oral contraceptives lower gonadotrophin levels [53].

The hormone-stimulation hypothesis suggests that excessive androgen stimulation of the OSE promotes neoplastic transformation, while progesterone is protective [53]. This is supported by the observed higher risk in conditions associated with elevated androgen levels such as PCOS, acne, and hirsutism, and the lower risk with gestation and use of oral contraceptives [50, 53]. Pregnancy is associated with increased progesterone levels [53]. Other than ovulation inhibition, protection conferred by oral contraceptives can be attributed to the progestogen component and a decrease in androgen levels [53, 55].

The epithelial group of ovarian tumours do not resemble cells normally found in the ovary but, rather, resemble cells of Müllerian origin. Endometrioid and clear-cell carcinoma resemble the endometrium, mucinous tumours the endocervix, and serous tumours the tubal epithelium [52, 56]. To explain this, it has been argued that the OSE first undergoes metaplasia into Müllerian type cells, followed by neoplastic transformation (the coelomic metaplasia theory) [52].

The fact that ovarian tumours do not bear resemblance to cells normally found in the ovary, and the fact that no precursor lesions have been identified within the ovary for type II tumours have led to questions regarding the origin of cancer cells [47, 48]. It has been postulated that epithelial ovarian tumours arise from extra-ovarian sites with secondary involvement of the ovary.

In the Müllerian-origin theory, pelvic serous carcinomas are thought to arise from the fallopian tube (tubal-origin theory). Pelvic or extra-uterine serous carcinoma is classified as serous ovarian carcinoma, fallopian tube serous carcinoma (FTSC), and primary peritoneal serous carcinoma based on the presumed site of origin. The criteria used for the classification of pelvic serous carcinomas are biased towards an over-diagnosis of ovarian serous carcinoma at the expense of tubal and peritoneal [48, 50, 51, 56]. The tubal-origin theory is based on histological findings of early lesions, known as serous tubal intraepithelial carcinomas (STICs), in the fallopian tubes of women with *BRCA1* or *BRCA2* mutations undergoing prophylactic surgery (bilateral salpingo-oophorectomy). STICs, mainly located in the distal end of the fallopian tube (fimbria), arise from secretory cells of the tubal epithelium (tubal epithelium has secretory cells and ciliated cells), are positive for *p53*, and are

considered precursor lesions to serous carcinoma [48, 50, 51]. STICs are also associated with pelvic serous carcinoma in patients with unknown *BRCA* mutation status [47].

STICs have also been found in association with ovarian and primary peritoneal high-grade serous carcinoma. In one study, 37% of cases with primary peritoneal serous cancer had STICs. This is in spite of the study being limited by variation in the degree of analysis of the fallopian tube (it is expected that extensive examination of the fallopian tube would have yielded a higher number of STICs). In addition, those with STICs were significantly older (P = 0.007) and more likely to have stage IV disease (P = 0.037) than those without STICs [51]. Age is a risk factor for epithelial ovarian cancer and HGSOC is usually diagnosed at an advanced stage. In studies where the fallopian tubes were comprehensively examined, using the Sectioning and Extensively Examining the Fimbriae (SEE-FIM) protocol[1][57], STICs were found in 50 to 60% of women with ovarian and peritoneal high-grade serous carcinoma without known genetic predisposition to ovarian cancer (non-familial ovarian cancer) [48]. No similar lesions have been found in the ovaries and no precursor lesions have been found in studies of contralateral ovaries of women with non-familial unilateral ovarian cancer [52]. Therefore, pelvic serous carcinoma is thought to arise from the fallopian tube, the ovary being a secondary site.

STICs have been found in direct continuity with short stretches of secretory cells with positive nuclear staining for p53, termed "p53 signatures"[47]. p53 signatures have also been found in the absence of STICs or cancer [52]. *p53* mutations have been reported in 57% of cases of p53 signatures [50, 52]. *p53* is a tumour suppressor gene located on chromosome 17. Its protein product, tumour protein 53 (TP53 also known as p53), halts the cell cycle to allow for repair of damaged DNA, or induces apoptosis (programmed cell death). Its cellular levels rise in response to DNA damage [58]. *p53* mutations are associated with many human cancers [45, 53, 58]. p53 signatures are more common in the fallopian tube epithelium as demonstrated by a study in which, out of 75 *BRCA* mutation carriers examined, 29 p53 signatures were found in the fallopian tubes, 1 in the OSE, and none in CICs [54].

The tubal-origin theory proposes that pelvic serous carcinomas arise from the distal tubal epithelium. The finding of p53 signatures in continuity with STICs has led to the conclusion that p53 signatures precede STICs [48]. With respect to the natural history of cancer, an ovarian carcinogenesis model has been proposed, starting from DNA damage, followed by

[1] SEE-FIM protocol increases the surface area of the fimbria under examination by 60%.

p53 signatures which progress to STICs, and finally to invasive cancer [50, 52]. Support for the link between STICs, p53 signatures, and invasive tubal carcinoma is by the demonstration that they arise from secretory cells, are mainly found on the tubal fimbriae, and when concurrent, express similar *p53* mutations [50, 51, 54]. In addition, STICs, HGSOC, primary peritoneal serous carcinoma, and FTSC have similar *p53* mutations in some cases [50]. Furthermore, the peritoneum is lined by mesothelium and therefore, malignant mesothelioma and not serous carcinoma is expected [51]. If serous carcinoma is of tubal origin, this would explain the advanced stage at diagnosis because the cancer cells, arising from the tube, have access to the entire peritoneal cavity [51, 52].

The prevalence of p53 signatures in non-neoplastic fimbria has been found to be the same in women with and without *BRCA* mutation, one third in each [47, 52]. This suggests that factors influencing p53 signatures are independent of *BRCA* mutation status. It is plausible that p53 signatures are a result of a physiological increase in TP53, in response to DNA damage [52, 54]. The observed higher risk of ovarian cancer in *BRCA* mutations carriers suggests that *BRCA* mutations influence progression of p53 signatures to STICs, and eventually invasive cancer [47].

Clear-cell carcinoma and endometrioid tumours have been associated with implants of endometriosis elsewhere in the pelvis and are, therefore, thought to arise from the endometrium [48, 52]. The cause of endometriosis, though not established, is thought to be mainly as a result of retrograde menstruation [23, 45]. Supporting the association with endometriosis is the observation that tubal ligation decreases the risk of clear-cell and endometrioid carcinoma but not other epithelial types [48]. In one study, the magnitude of risk reduction was found to be statistically significantly lower for serous than for clear-cell ($P = 0.0018$) or endometrioid ($P < 0.0001$) cancer [59]. In the Müllerian-origin theory, mucinous tumours are thought to arise from the endocervix. The Müllerian-origin theory is supported by the observed protection conferred by tubal ligation and hysterectomy, without oophorectomy [48]. This protection has also been attributed to blockage of carcinogens, from the external environment, from reaching the ovaries via the Müllerian tract [23, 45].

The coelomic metaplasia theory proposes that the OSE undergoes metaplasia under hormonal influence, with metaplasia being more likely in CICs, into Müllerian-type cells followed by neoplastic transformation [53, 56]. However, due to the intimate contact between the fallopian tubes and the ovary during ovulation, it is conceivable that dislodged normal tubal

epithelial cells can get incorporated into the disrupted OSE, forming inclusion cysts, which later undergo neoplastic transformation [48, 60]. Low-grade and high-grade serous carcinoma can then develop via different pathways [52]. In support of this theory are the findings of a study done by Li et al. in which a majority of ovarian sections examined (46 of 48 cases [96%]) were lined only by OSE of mesothelial phenotype; in contrast, 78% (667 of 856 cases) of epithelial inclusions were of fallopian tubal phenotype [60].

The Müllerian-origin theory is limited by the fact that women with *BRCA1* or *BRCA2* mutation who undergo prophylactic surgery are still at risk of peritoneal serous carcinoma [56]. Furthermore, mucinous tumours resemble the gastrointestinal mucosa and the origin of Brenner tumours, which resemble the urothelium, has not been explained [48, 52]. These findings suggest that there could be still other sources of cancer cells.

The secondary Müllerian-origin theory proposes that ovarian cancer arises from vestigial embryological remnants of the Müllerian system, which can act as a source for all the epithelial cell types of ovarian cancer. Microscopic vestigial embryological remnants lined by Müllerian epithelial cells (including endosalpingiosis, endocevicosis, and endometriosis), which are collectively referred to as the "secondary Müllerian system," are found in the paraovarian and paratubal areas and also within the ovarian cortex and medulla [56]. Brenner and mucinous tumours are associated with, and thought to arise from, nests of transitional-type epithelial cells: Walthard cell nests, located at the tubal-peritoneal junction (paraovarian and paratubal areas), and hence, their non-Müllerian appearance. Previously, mucinous tumours had been thought to originate from the endocervix but, due to their non-Müllerian appearance the cell-nest theory is preferred. Mucinous tumours more closely resemble gastrointestinal mucosa than endocervix [48, 52]. Since the secondary Müllerian system is also found within the ovary, albeit rarely, ovarian cancer can be viewed as originating from the ovary in this instance [56].

The theory of extra-ovarian origin of epithelial ovarian tumours is limited by the observed high levels of ovarian involvement. Although serous tumours are thought to arise from the tubal epithelium, serous tumours involve the ovaries and other pelvic organs more extensively than the tubes. In addition, endometrioid and clear-cell tumours are usually confined to the ovaries although endometriosis usually involves multiple sites in the pelvis. It has also been suggested that, rarely, LGSC progresses to HGSC [48]. This is due to the

finding of HGSCs in association with serous borderline tumours and LGSCs, with identical *KRAS* mutations and no *p53* mutations [52].

With the Müllerian- and secondary Müllerian-origin theories, the germ-cell and gonadal stromal tumours are the only tumours thought to be of true primary ovarian origin [48].

Both theories suggest that both type I and type II tumours arise from extra-ovarian sites with secondary involvement of the ovary. Adoption of this concept will have implications for prevention, screening, and treatment of ovarian cancer. It may also influence nomenclature of ovarian tumours, and impact on future research directions.

Prophylactic surgery for women with familial predisposition to ovarian cancer may be limited to salpingectomy or fimbriectomy [48, 50, 51, 56], sparing the ovaries, and therefore, preserving fertility (this can be achieved through assisted reproductive technology) and avoiding early menopause with its attendant risks such as osteoporosis, vasomotor instability, and an increased incidence of cardiovascular diseases [45]. In addition, if the tumour arises from the tubal fimbria, fimbriectomy could be an option for patients who desire tubal ligation as a method of contraception, while warning on the irreversibility of the procedure, as this would further decrease the risk of ovarian cancer [50]. Knowledge of the pathogenesis of ovarian cancer will also help in understanding the mechanism of the risk factors and, therefore, aid in the development of effective preventive strategies [56].

Based on the view that epithelial ovarian cancer arises from OSE, screening has focused on early detection of ovarian cancer while it is still confined to the ovary. With appreciation of the dualistic model of pathogenesis, one screening test may not detect all the different types of ovarian cancer. Type I tumours, which account for 25% of ovarian cancer and 10% of deaths, are slow growing, and can be detected at an early stage by pelvic examination, and transvaginal ultrasound (TVS). Therefore, development of a screening biomarker is not urgent for this group of tumours. Future research on screening should be more focussed on type II tumours, which are rapidly progressing and account for 75% of the disease burden and 90% of deaths from ovarian cancer. The aim of screening should be to detect the tumour at low-volumes, rather than at an early stage, by developing sensitive and specific biomarkers that are expressed early in ovarian carcinogenesis [48]. It is also important to determine the time interval between the development of STIC and peritoneal spread and to develop tests with high sensitivity and specificity for detecting STICs [47].

In addition, different therapeutic approaches should be used for type I and type II tumours. Surgery is generally effective for type I tumours as they are usually detected at an early stage. At an advanced stage, type I tumours, due to their slow growing nature, may not be responsive to the chemotherapeutic agents that are effective for the rapidly proliferating type II tumours [48]. A different therapeutic approach is, therefore, needed for advanced type I tumours. Surgery in type II tumours, HGSOC, is usually limited to debulking due to the fact that they are usually detected at an advanced stage.

Staging of ovarian cancer may also be affected, with proposals of staging according to tumour bulk [52]. In addition, various names have been suggested in place of ovarian cancer, including "extra-uterine Müllerian carcinoma", to reflect the origin of the tumour cells. Pelvic serous carcinomas arise from the fallopian tube (tubal-origin theory) and should therefore be treated as one disease entity [56].

All said, it is important to note that the true pathogenesis of ovarian cancer has not been established. Understanding the pathogenesis of a disease is important in the development of effective screening and therapeutic methods, as well as preventive strategies. Currently, there is no effective screening method for ovarian cancer and current therapeutic approaches have had little success. Nonetheless, there has been progress in prevention, with oral contraceptives having been shown to be effective in significantly decreasing the risk of ovarian cancer. Consequently, emphasis should be placed on prevention.

2.5 RISK FACTORS AND PROTECTIVE FACTORS

2.5.1 AGE

Age is a known risk factor for ovarian cancer. The incidence of ovarian cancer increases from about 4 per 100,000 in 30-35 year-old women to about 40 per 100,000 in those aged 60-64 years. Only 10-15% of epithelial ovarian tumours occur in pre-menopausal women [61]. In a prospective cohort study (Nurses' Health Study [NHS]), overall, the incidence of ovarian cancer increased with age; however, the rate of increase was significantly slower in post-menopausal women (2.5% per year) compared to pre-menopausal women (11.6% per year) [46]. Changes which occur with age that may impact on risk of ovarian cancer include: ovarian depletion of oocytes which eventually leads to menopause, decreased production of steroid hormones, and increased gonadotrophin production [61].

2.5.2 REPRODUCTIVE FACTORS

2.5.2.1 Parity and timing of pregnancy

Parity is a well-established protective factor against ovarian cancer, with higher numbers of births providing better protection. In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, ever, - compared to never, -having had a child was associated with a risk reduction of 29% (HR = 0.71; 95% CI = 0.59-0.87). An inverse relationship was observed between number of births and risk of ovarian cancer (P-trend = 0.03). Each additional term delivery was associated with an 8% decrease in risk (HR = 0.92; 95% CI = 0.85-0.99) [62]. Similarly, in a hospital-based case-control study in China, compared to women with ≤1 birth, those with ≥3 births experienced a 57% lower risk of ovarian cancer (OR = 0.43; 95% CI = 0.30-0.62). A significant trend was also observed (P <0.001) [63].

The inverse association with parity extends to women at high risk of ovarian cancer: *BRCA1* mutation carriers with history of one or more live births have been shown to have a lower risk of ovarian cancer (HR = 0.41; 95% CI = 0.18-0.94; P = 0.03) compared with *BRCA1*mutation carriers who were nulliparous. *BRCA1* mutation carriers with ≥4 live births had a greater reduction in risk (HR = 0.15; 95% CI = 0.04-0.56; p = 0.005), although no statistically significant trend was observed with higher parity (P-trend = 0.1). No association with parity was observed in *BRCA2* mutation carriers (all P ≥0.3) [64]. Multiple pregnancies have also been shown to have a stronger inverse association with ovarian cancer than singleton pregnancies; among parous women, history of multiple births was associated with an OR of 0.71 (95% CI = 0.53-0.95) [65].

There is evidence that the sex of offspring affects the association between parity and risk of ovarian cancer. In a population-based case-control study, compared to giving birth to girls only, having all boys was associated with an OR of 0.80 (95% CI = 0.58-1.10). A stronger inverse association was observed in women with both girls and boys (OR = 0.58; 95% CI = 0.43-0.79). However, no trend was observed between having boys or girls and the risk of ovarian cancer in multiparous women and there was no relationship with birth order of the different sexes. However, due to the small numbers, the findings may have been due to chance [66]. The association with sex may vary across the different histological types of ovarian cancer. Jordan et al. found a statistically significant higher risk of mucinous ovarian cancer (both invasive and borderline) in women who had given birth to boys only over those with girls only (OR = 2.19; 95% CI = 1.15-4.17), with a clear trend of higher risk with higher

number of male offspring (P-trend = 0.003). No relationship between sex of offspring and the risk of other histological subtypes of epithelial ovarian cancer was observed [67].

Timing of pregnancy has also been shown to affect risk. Milne et al. reported a significant linear trend (adjusted for age) of age at first birth and risk of ovarian cancer in both *BRCA1* and *BRCA2* mutation carriers (HR = 0.65; 95% CI = 0.52-0.83; P <0.001; per 5 years age) [64]. In yet another study, compared to women who had their first pregnancy when they were ≥25 years, those who had their first pregnancy at ≤19 years were at a higher risk of ovarian cancer (OR = 1.4; 95% CI = 1.1-1.8)-[adjusted for parity] [68]. The association with age at last delivery is stronger than that with age at first delivery. In an Australian case-control study, age at last birth was inversely associated with ovarian cancer regardless of the age of the woman and more important than parity, age at first delivery, or time since last delivery. Risk of ovarian cancer decreased by 3% (95% CI = 1-6%) with each year greater age at last birth. No association with age at first birth after adjustment for parity and age at last delivery was observed [69]. A decrease in the strength of association with time since last delivery has also been observed [68]. In a nested case-control study done in Sweden no association with birth spacing was observed [70].

The association between disrupted pregnancies and the risk of ovarian cancer is unclear [71, 72]. Chen et al. assessed the impact of abortion in terms of nulliparous and parous women separately, spontaneous and induced abortions, cumulative duration of pregnancies ending in abortion, or whether the abortion preceded or occurred after a first delivery, and concluded that abortions either were not associated with the risk of ovarian cancer or the risk was too small for reliable detection in epidemiologic studies [71]. On the other hand, Jordan et al. reported a higher risk of ovarian cancer in parous women with history of preterm births compared to those with only full-term births (OR = 1.48; 95% CI = 1.02-2.15) [67].

Although parity reduces the incidence of breast, ovarian, and endometrial cancer, parous women are at increased risk of diabetes mellitus, gallbladder disease, cancer of the cervix, and a number of circulatory diseases (ischaemic heart disease, hypertension and cerebrovascular disease). A study done in England and Wales involving 12 million women aged 45-74 years, reported a 20% higher overall mortality in the period 1959-1960 in parous compared to nulliparous women. This was mainly accounted for by a higher incidence of circulatory diseases in parous women compared to nulliparous women [73].

2.5.2.1.1 Possible mechanisms

The mechanism of the inverse association with pregnancy is unknown. Parity is thought to confer protection by ovulation inhibition, reduced levels of serum gonadotropins, decreased inflammation and changes in the plasma levels of steroids [66]. High levels of progesterone may also be relevant, perhaps by clearing the ovary of pre-malignant cells [65]. Pregnancy also prevents substances from the vagina and perineum that are potentially carcinogenic - as well as growth factors from the uterus - from reaching the ovary [62].

Apoptotic clearance of malignant epithelial cells, mediated by high levels of progesterone in pregnancy, may explain the decrease in the level of protection with increase in time since last pregnancy [68, 69]. This is because longer time allows accumulation of mutations by epithelial cells [69]. This also explains the stronger association with last delivery at older age compared to younger age: at an older age there is likely to be a larger pool of cells that have undergone malignant transformation [69].

Changes in the hormonal milieu during the course of pregnancy and differences in hormonal concentrations between pregnancies may explain the differential risk of ovarian cancer observed with different pregnancy outcomes [29, 67]. Levels of progesterone are almost similar to the luteal phase of the menstrual cycle in early pregnancy (up to the 13^{th} week) with significant increase thereafter [29]. Highest levels of progesterone are experienced in the third trimester [69]. There is rapid rise of oestradiol concentration in the first 13 weeks of pregnancy; by 8 weeks, they are above the normal menstrual-cycle levels, with a gradual rise thereafter. A return to normal pre-pregnancy levels of oestrogen and progesterone is seen by 1 year post-delivery [29]. Androgen levels are relatively constant throughout pregnancy and are almost the same before, during, and after pregnancy [29].

The effect of a disrupted pregnancy on ovulation inhibition and hormonal levels may not have a detectable effect on the risk of ovarian cancer [71, 74]. A higher number of ovulations are prevented by term than preterm pregnancies. In addition, the highest levels of progesterone (which is responsible for clearance of abnormal cells) are experienced in late pregnancy [67].

Serum concentrations of oestriol and hCG are lower in women bearing male foetuses compared to those with female foetuses, perhaps explaining the higher protection observed in some studies with male offspring [66, 67].

Greater inverse association with multiple, compared to singleton, pregnancies is at odds with the incessant ovulation and gonadotropin hypotheses. This is because women with history of multiple births have a higher frequency of double-ovulation and higher levels of gonadotropins during their reproductive years, hence might be expected to be at a higher risk of ovarian cancer [65]. Higher levels of progesterone in multiple, compared to singleton, pregnancies may be responsible for the stronger inverse association with the former [29, 65]. Conversely, findings of no difference between singleton and multiple pregnancies may be attributable to the fact that multiple pregnancies are more likely to result in preterm births than singleton pregnancies [67].

2.5.2.2 Breastfeeding

An inverse association between breastfeeding and the occurrence of ovarian cancer has been reported in many studies, with longer duration of breastfeeding associated with more markedly lower risk [75-78]. This inverse association has been shown to be independent of parity [75-78]. In the Australian Ovarian Cancer Study, compared to those who had never breastfed, breastfeeding was associated with a RR of 0.77 (95% CI = 0.61-0.96), with a clear trend of decreasing risk with increasing duration of breastfeeding. Each month of breastfeeding was associated with 1.4% (95% CI = 0.6-2.2%) lower risk of ovarian cancer. These findings were independent of parity. Breastfeeding for more than 12 months after delivery was not associated with any additional reduction in risk [78]. Similarly, in a pooled analysis of data from two prospective cohort studies (NHS and NHS II), comparable findings were observed. Compared to never breastfeeding, ever breastfeeding was associated with a statistically non-significant lower risk of ovarian cancer (RR = 0.86; 95% CI = 0.70-1.06). However, breastfeeding for ≥18 months was associated with a statistically significantly lower risk (RR = 0.66; 95% CI = 0.46-0.96). An RR of 0.98 (95% CI = 0.97-1.00) was observed for each month of breastfeeding. The findings were not modified by parity or use of oral contraceptives [76]. In these two studies for obvious reasons, analysis of the association with breastfeeding was confined to parous women.

In contrast, a hospital-based case-control study in Italy observed no statistically significant decreasing trend in risk with increase in duration of breastfeeding or number of children breastfed when parity and other confounders were taken into account [79].

In a meta-analysis of all published studies through December 2012 that included the above three studies, ever-breastfeeding was associated with a 24% lower risk of ovarian cancer (RR

= 0.76; 95% CI = 0.69-0.83), compared to never-breastfeeding. Every five months of breastfeeding was associated with an 8% lower risk (RR = 0.92; 95% CI = 0.90-0.95). All studies appropriately restricted the analysis to parous women [77].

2.5.2.2.1 Possible mechanisms

The inverse association with breastfeeding may be explained by suppression of ovulation [76, 77, 79, 80] and decreased serum levels of gonadotropins [76, 77]. Ovulation occurs within six weeks after delivery in women who do not breastfeed [76]. Anovulation in breastfeeding women occurs as a result of elevated serum FSH and prolactin levels and a decrease in LH levels [80]. Ovulation inhibition is partly reliant on frequency and duration of breastfeeding. As a result, ovulation resumes around the time introduction of solid food starts (around 6 months) which may explain the lack of additional protection with breastfeeding for >12 months [78].

2.5.2.3 Ovulation

The incessant-ovulation hypothesis was first proposed by Fathalla in 1971. This was informed by the observations that the frequency of ovulation and the incidence of ovarian cancer are higher in women than animals and that this difference is mainly due to a higher incidence specifically of epithelial ovarian cancer in women. Secondly, epithelial tumours are very rare in the pre-pubertal period and in women with gonadal dysgenesis, whereas non-epithelial tumours occasionally develop. In addition, nulliparous women are at higher risk of ovarian cancer than parous women [81].

Following this, studies have been done to assess the association between the risk of ovarian cancer and lifetime ovulatory cycles. In an Australian population-based case-control study, each additional year of ovulation was associated with a 6% higher risk of ovarian cancer (OR = 1.06; 95% CI = 1.04-1.08) after adjusting for age. The highest risk was associated with ovulations that occurred between 20 and 29 years of age (OR = 1.20; 95% CI = 1.13-1.27). Risk was similar for both invasive and borderline tumours. The increase in risk was confined to non-mucinous tumours; no effect on the risk of mucinous tumours was observed (OR = 1.01; 95% CI = 0.98-1.04). There was statistically non-significant difference in risk according to cause of anovulation (P-heterogeneity = 0.53). An OR of 0.88 (95% CI = 0.78-0.98) for each year of anovulation due to childbirth was observed; likewise, for OC use, OR = 0.92 (95% CI = 0.89-0.94); and for abortions, OR = 1.35 (95% CI = 0.73-2.48). Although abortion seemed to be associated with a 35% higher risk, which is at odds with the expected effect of anovulation, the confidence interval was wide [82].

Similarly, in an Italian hospital-based case-control study, each year of anovulation was associated with a 2.5% lower risk of ovarian cancer (OR = 0.975; 95% CI = 0.965-0.985). Difference in association according to the cause of anovulation was observed. For each year of ovulation avoided, the strongest associations (OR = 0.92; 95% CI = 0.87-0.97, and OR = 0.91; 95% CI = 0.87-0.95) were seen for OC use and parity respectively; age at menopause was associated with an OR of 0.97 (95% CI = 0.95-0.98), whereas abortion (OR = 0.90; 95% CI = 0.76-1.06) and age at menarche (OR = 0.99; 95% CI = 0.96-1.03) showed no association [83].

Consistent with the above studies, a population-based case-control study reported a statistically significant positive association between total ovulatory (log) years and the risk of ovarian cancer (OR = 1.78; 95% CI = 1.24-2.57). A positive association was found for premenopausal women but not for postmenopausal women (OR = 2.49 and OR = 0.88, respectively; P-interaction = 0.006), with the strongest association again seen with ovulations occurring in the 20-29 years of age period. The association with use of oral contraceptives, pregnancy, breastfeeding, and lifetime ovulation were statistically significant for women who were pre-menopausal at diagnosis but not post-menopausal women, although there was no statistically significant interaction [80].

Furthermore, in a prospective cohort study (NHS), duration of ovulation (years of menstruation minus anovulatory years) was positively associated with ovarian cancer. Each year of ovulation was associated with an increase in risk of 9.6% (95% CI=8.1-11.1%). Increase in risk associated with ovulatory years was slower after menopause (increase in risk per year of ovulation = 2.6%; 95% CI = 0.8-4.4%) [46]. However, the study included only parity and oral-contraceptive use in calculating anovulatory years and did not consider cycle length.

In summary, total ovulatory years are positively associated with the occurrence of ovarian cancer. Ovulations occurring in the 20-29 years of age period have the highest association with risk. The association with ovulation varies across the different histological types, being more apparent for non-mucinous than mucinous tumours. Congruent with this, factors that lead to anovulation show inverse associations with ovarian cancer. The magnitude of the inverse association afforded by ovulation suppression differs according to the cause of anovulation.

2.5.2.3.1 Possible mechanisms

Incessant ovulation is thought to increase the risk of ovarian cancer by repeated disruption and repair of the ovarian surface, accompanied by proliferation of cells to repair the defect, which may lead to DNA damage; entrapment of epithelial cells in the stroma; and high levels of hormonal exposure at the time of ovulation [84]. The observation that ovulations that occur in the 20-29 years of age period have the strongest association with risk may be because this is the period that anovulation due to reproductive events and OC use occurs most commonly. In addition, it may be due to a long latency period of ovarian cancer from initiation (initiation by incessant ovulation in this case) to clinical manifestation of disease. If ovulation had a promotional effect, higher risk would have been seen with ovulatory cycles in older age-periods (risk as a result of ovulatory cycles). The peak of ovarian cancer incidence is the 60-70 years age-period [82]. The difference in the strength of the inverse association with anovulation between premenopausal and postmenopausal women may be explained by a rise in serum concentration of gonadotrophins in the premenopausal period. Plasma FSH levels increase with age with a steep rise after the perimenopause; oestradiol decreases around menopause. In addition, anovulatory cycles are more common in the perimenopausal period; therefore, ovulation suppression would be less effective in decreasing the risk of ovarian cancer during this period [80]. The lack of uniformity in the association with the different causes of anovulation points to mechanisms of carcinogenesis of ovarian cancer other than ovulation suppression [83]. Oral contraceptives have a greater effect in reducing levels of gonadotrophins than parity and this may explain the difference in the strength of inverse association between these two exposures [80].

Suppression of mucin (MUC)1-associated specific immunity has been proposed as an immunologic basis of the effect of incessant ovulation on ovarian, breast, and endometrial cancer. In a study done in the USA (New Hampshire and Massachusetts), number of ovulatory years was inversely associated with the presence of anti-MUC1 antibodies and was positively associated with a higher risk of ovarian cancer (P for trend <0.001). Repetitive events that promote expression of anti-MUC1 antibodies may promote anti-MUC1 immunologic memory and, as a result, decrease the risk of ovarian cancer. Such events include tubal ligation and use of oral contraceptives, which are also inversely associated with ovarian cancer. Association of incessant ovulation with decreased anti-MUC1 antibodies may be the result of damping of MUC1 specific immune response or the absence of events (anovulation factors/others e.g tubal ligation) which would otherwise promote the expression

of anti-MUC1 antibodies. The presence of anti-MUC1 antibodies, although they may have a protective role against ovarian cancer, does not necessarily imply that it has a clearing effect on premalignant cells; it may rather be a surrogate for other immune mechanisms. It may also act by promoting other antigens expressed by epithelial cells [85]. MUC1 is a high molecular weight glycoprotein that is in the same family as CA-125 [85, 86]. MUC1 is normally present in the epithelial cells of the breast ducts and the respiratory, genitourinary, and digestive tracts [85, 86], but not the ovarian surface epithelium [85]. Ovarian, endometrial, and breast cancers overexpress MUC1, which may lead to increased production of anti-MUC1 antibodies [85, 86]. Expression of MUC1 and anti-MUC1 antibodies may also occur in healthy men, as well as in women particularly during pregnancy and breastfeeding [86].

2.5.2.4 Menstrual pattern and ages at menarche and at menopause

Findings regarding the relationship between ages at menarche and at menopause and the risk of ovarian cancer have been inconsistent [46]. In the NHS, late age at menopause was associated with higher risk of ovarian cancer (risk increase = 62%; 95% CI = 36-96%, for menopause at age 55 compared to 45) and late age at menarche was inversely associated (risk reduction = 31%; 95% CI = 27-34%, for menarche at 15 years compared to 11 years) [46]. In contrast, a population-based case-control study observed no relationship between ages at menarche and at menopause and menstrual regularity with the risk of ovarian cancer [87]. Pelucchi et al. observed no association between ages at menarche and at menopause with the risk of ovarian cancer or with length of menstrual cycle [83]. Compared to women with cycle lengths of 26-30 days, women with menstrual cycles of <21 day and ≥35 days had an OR of 0.90 (95% CI = 0.53-1.54) and 0.57 (95% CI = 0.30-1.08), respectively [83].

The lack of association between age at menarche and at menopause with the risk of ovarian cancer may be because the first and last menstrual period may not accurately reflect the initiation and cessation of ovulation, whereas pregnancy and OC use are a more accurate reflection of anovulation [87].

2.5.3 INFERTILITY AND USE OF OVULATION-INDUCING DRUGS

Several studies have observed a statistically significant higher risk of ovarian cancer in women with a diagnosis of infertility [88-91]. In a prospective cohort study in Denmark, which involved 54,362 women diagnosed with infertility, compared to the general population, a statistically significant higher risk of ovarian cancer was observed in women with a diagnosis of infertility (unadjusted for parity: standardised incidence ratio [SIR] = 1.69; 95% CI = 1.44-1.98, adjusted for parity: SIR = 1.46; 95% CI = 1.24-1.71). This was

mainly attributed to a higher risk of serous tumours (SIR = 2.01; 95% CI = 1.60-2.49). A statistically non-significant inverse association with mucinous tumours was observed (SIR = 0.65; 95% CI = 0.30-1.23) [89]. Similarly, in a pooled analysis of eight case-control studies, history of subfertility was associated with a higher risk of ovarian cancer (OR = 1.26; 95% CI = 1.14-1.39), with an equal point estimate for ever- and never-pregnant women (OR = 1.2). Higher risk was observed with longer duration of trying to get pregnant (OR = 2.7; 95% CI = 1.9-3.7, for ≥5 years vs <1 year). In addition, a statistically significant higher risk of ovarian cancer was observed in women with infertility due to endometriosis (OR = 1.73; 95% CI = 1.10-2.71) and those with infertility of unknown cause (OR = 1.19; 95% CI = 1.00-1.43) [88].

Some studies failed to demonstrate an association between infertility and the occurrence of ovarian cancer in both users and non-users of fertility drugs [92, 93]. In contrast, a statistically significant higher risk was observed in women with infertility of unknown cause (SIR = 4.59; 95% CI = 1.91-11.0) [93] and in women who used fertility drugs and failed to conceive (OR = 3.13; 95% CI = 1.01-9.67) [92].

A number of studies, while reporting a positive association between infertility and the incidence of ovarian cancer, have shown no difference in risk between users and non-users of fertility drugs [88, 90, 94]. In a retrospective cohort study of 12,193 women evaluated for infertility in USA (median length of follow-up = 18.8 years), infertility was associated with a statistically significant higher risk of ovarian cancer (SIR = 1.98; 95% CI = 1.2-2.6). There was no difference in risk between those who did and those who did not use fertility drugs (clomiphene or gonadotropins). After adjusting for parity, the rate ratio for ever-use of clomiphene was 0.8 (95% CI = 0.4-1.5), and that for gonadotropins was 1.1 (95% CI = 0.4-2.8). No statistically significant association was observed with longer duration of use of fertility drugs or with greater numbers of cycles of clomiphene. Dose of clomiphene did not influence risk nor did type of infertility. No statistically significant association was observed in women who used clomiphene but did not conceive (RR = 1.7; 95% CI = 0.5-5.7 based on only 6 cases) nor in women who conceived (RR = 0.8; 95% CI = 0.3-1.8). Compared to the general population, women in this study had a standardised mortality ratio (SMR) of 1.9 (95% CI = 0.9-3.5). No difference in mortality from ovarian cancer was observed between users and non-users of fertility drugs [90].

Inconsistent findings for ovarian cancer and ever-use of infertility drugs have also been observed [91]. In a prospective cohort study of 3,837 women evaluated for infertility, among

women with infertility, use of clomiphene was associated with a higher risk (RR = 2.3; 95% CI = 0.5-11.4). Use of clomiphene for <12 monthly cycles was not associated with a higher risk, whereas use for ≥12 cycles was associated with a statistically significantly elevated risk of ovarian cancer (RR = 11.1; 95% CI = 1.5-82.3) but with a very wide confidence interval. In contrast, use of human chorionic gonadotropin was not associated with risk of ovarian cancer (RR = 1.0; 95% CI = 0.2-4.3). The risk of ovarian cancer was statistically significantly higher in this cohort of women than in the general population (SIR = 2.5; 95% CI = 1.3-4.5). The risk of borderline tumours was higher than that of invasive tumours (SIR = 3.3; 95% CI = 1.1-7.8 versus SIR = 1.5; 95% CI = 0.4-3.7) [91]. In the study by Ness et al., ever-use of fertility drugs was not associated with overall ovarian cancer risk. Among the ever-users, an OR of 1.60 (95% CI = 0.90-2.87) was observed in those who were never pregnant and an OR of 0.82 (95% CI = 0.62-1.09) in those who were ever pregnant. There was also no relationship with duration of use or the type of drug that was used longer (human menopausal gonadotropin or clomiphene citrate). Among never-pregnant women, a statistically significant association was observed with borderline serous tumours (OR = 2.43; 95% CI = 1.01-5.88) but not with serous invasive or other histological types. In ever-pregnant women, use of fertility drugs did not have a statistically significant association with any histological type [88].

2.5.3.1 Possible mechanisms

A common genetic predisposition to both cancer and infertility [89, 95], or the effects of infertility drugs [89] have been proposed as possible causes of the observed increase in risk. In addition, the underlying cause of infertility, for instance endometriosis, may also increase the risk of ovarian cancer [88].

Ovulation-inducing drugs are used in the treatment of infertility to induce multiple folliculogenesis (superovulation) [93, 96], the most commonly used drugs being clomiphene citrate and gonadotropins [96]. Of concern regarding the risk of ovarian cancer is the use of gonadotropins and multiple ovulations [92, 96, 97], which, according to the gonadotropin and incessant-ovulation hypothesis would result in an increase in the risk of ovarian cancer [92, 97]. There is also a rise in the serum levels of oestrogen (E_2) and progesterone which are also thought to influence the risk of ovarian cancer [96, 97].

The observation that use of infertility drugs may have a greater effect on the risk of developing benign ovarian tumours is thought to be due to a growth-promoting effect of

fertility drugs on pre-existing highly differentiated tumours [97]. On the other hand, increase in risk may also be the result of an impact earlier in the carcinogenesis process, as suggested by a study in which a statistically significantly higher dysplasia score was observed in women who had used ovulation-inducing drugs compared to parous women (7.92 vs 5.70; P = 0.012). There was no difference in the dysplasia score of nulliparous and parous women before and after adjustment for age and duration of oral contraceptive use (P = 0.85 and P = 0.87 respectively) [95].

The increase in risk observed with infertility or use of fertility drugs may also be attributed to surveillance bias. However, contrary to this hypothesis, Rossing et al. reported a mean follow-up time to diagnosis of 6.9 years; further nine of the 11 tumours diagnosed were detected after the end of follow-up, effectively ruling out surveillance bias [91].

Ovulatory drugs were introduced in the 1960s; the lack of a clear relationship between use of fertility drugs and the risk of ovarian cancer in studies done to date may be because an adequate number of women exposed to infertility drugs have not reached the age at which the incidence of ovarian cancer is at its highest [97]. This may also be due to methodological limitations [89, 90, 97]. Some studies did not control for parity and OC use, which are protective factors, and endometriosis, which is associated with higher risk; this may have biased the results [92]. Additional limitations include small number of cases, short follow-up time, and reliance on self-reported use of infertility drugs.

2.5.4 INFLAMMATORY FACTORS

2.5.4.1 Talcum

Talc and asbestos are thought to be ovarian cancer carcinogens [98]. Use of talcum powder has been associated with risk of ovarian cancer [98-100], with higher risk seen with use in perineal compared to non-perineal areas [99-101]. In a population-based case-control study in Los Angeles County, a statistically significant higher risk of ovarian cancer was observed in ever-users of talc (RR = 1.53; 95% CI = 1.13-2.09) and a lesser risk for use in non-perineal areas only (RR = 1.43, 95% CI = 1.03-1.98) [100]. In another population-based case-control study, compared to never use, use of talc in the perineal area was associated with a statistically significantly higher risk of EOC (OR = 1.58; 95% CI = 1.16-2.16), whereas use in non-genital areas was not associated with higher risk (OR = 1.08; 95% CI = 0.77-1.50) [99]. No dose-response relationship has been observed overall [99, 101], but a dose-response relationship confined to use of talc before the year 1975 has been reported [100]. Several

studies have observed a stronger association with serous histologic subtype than with other subtypes [98-101]. In one study, the greatest risk was observed for invasive serous tumours (OR = 1.70; 95% CI = 1.22-2.39) and no statistically significant association for mucinous tumours (OR = 0.79; 95% CI = 0.44-1.40) - both invasive and borderline. In contrast with these studies, a hospital-based case-control study reported no association between use of talcum and the risk of EOC (OR = 0.92; 95% CI = 0.24-3.62); risk did not differ by method of application (sanitary napkin, genital area, or thigh area), histological type, history of tubal ligation or hysterectomy, or duration of use [102].

Tubal ligation is thought to block contaminants from the lower genital tract from reaching the ovaries and peritoneum [103, 104]. In a study in which assessment of the relationship between use of perineal talc and EOC was restricted to use prior to hysterectomy or tubal ligation, an OR of 1.17 (95% CI = 1.01-1.36) was observed. Use of talcum after tubal ligation or hysterectomy was not associated with risk, irrespective of duration of use (P-trend = 0.61) [101]. In addition, in order to assess the effect of asbestos contamination of talcum prior to 1976, older and younger women were assessed separately. Higher risk occurred in both groups; therefore, the effect of talc use is not explained simply by the presence of asbestos [101].

2.5.4.1.1 Possible mechanisms

The finding of talc particles in ovaries of patients with ovarian cancer and with benign ovarian neoplasms, as well as demonstration of ovarian hyperplasia in guinea pigs and rabbits exposed to talcum powder triggered the suspicion that talc may cause ovarian cancer [102]. Talcum may cause ovarian cancer via chronic inflammation [99, 101], although its mechanism of carcinogenesis has not yet been established [99]. The strong relationship observed between serous ovarian cancer and talc use is probably due to the similarity between serous tumours and mesothelioma which is caused by asbestos, and the similarity in chemical properties between talcum and asbestos [101]. Lack of a dose-response relationship between talc use and the risk of ovarian cancer may be explained by the observation that there is no relationship between level of exposure to talc and amount of talc in normal ovaries. This suggests that a small threshold amount of talc may be required to cause an increase in the risk of ovarian cancer [101].

The credibility of talc as a cause of ovarian cancer is limited by the lack of a dose-response relationship. This may be due to the difficulty in defining what constitutes talcum application

[99]. The other limitation to the biologic credibility of talc use is the pattern of inconsistent findings [102]. This may be due to the lack of assessment of risk associated with talc use in relation to genital tract integrity [99, 102]. A lack of increase in risk in women with prior history of tubal ligation or hysterectomy has been demonstrated [101]. Furthermore, studies of talc before 1976 may have been confounded by the presence of asbestos contamination [98]. However, no difference in risk was observed between younger and older women [101].

2.5.4.2 PID

Pelvic inflammatory disease (PID) involves inflammation of the endometrium, fallopian tubes, and ovaries [87]. *Neisseria gonorrhoea* and *Chlamydia trachomatis* are responsible for 25-75% of PID [105]. Studies have had conflicting results regarding the association between the risk of ovarian cancer and PID [105]. In a population-based case-control study in Canada, self-reported history of PID was associated with higher risk of ovarian cancer (OR = 1.53; 95% CI = 1.10-2.13; P = 0.012). A higher risk with higher number of PID episodes was also observed (OR = 1.88 for ≥2 episodes). Lifetime prevalence of PID among controls was 18.1%; using this and the OR point estimate of 1.53, the authors estimated the population attributable risk of ovarian cancer due to PID to be 8.8% (95% CI = 1.8-17%) [106]. In contrast, the Australian Ovarian Cancer Study (an Australian nationwide population-based case-control study), found no statistically significant association between PID and the risk of ovarian cancer (OR = 1.15; 95% CI = 0.85-1.57). The risk did not differ by histologic type or time since diagnosis [101]. Information on PID in both studies may not be accurate because it was not verified using medical records.

Similar to the inconsistency in the results of the above studies, a population-based case-control study reported a positive association between the risk of ovarian cancer and serum chlamydia antibody titres (OR = 1.3; 95% CI = 1.0-1.6) in its initial results (117 cases, and 170 controls). The risk of ovarian cancer was 90% higher in women with the highest level of chlamydia antibodies compared to the lowest [105]. In a subsequent re-analysis using a larger sample (521 cases and 766 controls), a diametrically opposite relationship was reported: cases had a lower level of serum chlamydia antibodies than controls (OR = 0.6; 95% CI = 0.4-0.9; P-trend = 0.001) [107].

2.5.4.2.1 Possible mechanisms

PID can cause chronic inflammation, which may lead to DNA damage, increased cell proliferation, and increased production of cytokines, prostaglandins and growth factors, which together may lead to an increased risk of malignant transformation of cells [87]. PID

also leads to infertility in 10-20% of affected women. The resultant infertility or low parity may then contribute to the observed increase in the risk of ovarian cancer [105, 106].

The prevalence of PID in epidemiologic studies may be underestimated, which may bias the results towards the null. This is because PID is at times asymptomatic, may escape the attention of the patient, and is therefore underdiagnosed. In addition, there is the risk of under-reporting due the negative social connotations associated with the condition [105]. There is also the risk of recall bias in case-control studies. The difference in findings of the relationship between ovarian cancer and chlamydia antibodies within the same study could reflect unreliability of chlamydia antibodies as a marker of PID. In addition, the test does not detect gonorrhoea which also causes PID; further about 40% of affected women do not develop detectable antibodies. There is also a decline in chlamydia antibody titres over time [107].

2.5.4.3 Analgesics

Use of nonsteroidal anti-inflammatory drugs (NSAIDs) has been associated with a decreased incidence of colorectal cancer [27, 108], and is also associated with lower stage at diagnosis of breast and prostate cancer [27]. Studies assessing the relationship between use of NSAIDs and the occurrence of ovarian cancer have reported an inverse association [108-110], no association [101], and a positive association [100, 111]. Use of NSAIDs has been associated with lower risk of ovarian cancer of up to 40%. In the NHS, a prospective cohort study involving 76,821 women with a follow-up of 12 years (1,222,412 person-years), ever-use of NSAIDs was inversely associated with the occurrence of ovarian cancer (RR = 0.60; 95% CI = 0.38-0.95) [112]. Relationships with duration of use, frequency of use, and timing of first and last use of NSAIDs have been inconsistent [108, 110, 112, 113]. There is also evidence of an inverse association between the incidence of ovarian cancer and use of paracetamol (a non-NSAID analgesic) [108, 110]. Cramer et al. reported an OR of 0.52 (95% CI = 0.31-0.86) associated with regular use of paracetamol for ≥6 months [108]. Some studies observed no association between the use of paracetamol and the risk of ovarian cancer [113].

Conversely, aspirin, other NSAIDs, and paracetamol have also been associated with higher risk of ovarian cancer [100, 111]. In a population-based case-control study in Washington state, use of aspirin, paracetamol, or any NSAIDs (including aspirin) for ≥5 times/month for >10 years was associated with a statistically significant higher risk of ovarian cancer (OR = 1.6; 95% CI = 1.1-2.2, OR = 1.8; 95% CI = 1.3-2.6, and OR = 1.3; 95% CI = 1.0-1.7,

respectively). In somewhat puzzling contrast, recent use of aspirin (within the last five years) was associated with a lower risk of ovarian cancer (OR = 0.6; 95% CI = 0.4-1.0) [111]. In addition, a population-based case-control study in Los Angeles County, which strangely included paracetamol in the definition of NSAIDs, reported a 25% higher risk of ovarian cancer per five years of use of NSAIDs (RR = 1.25; 95% CI = 1.10-1.42) [100].

2.5.4.3.1 Possible mechanisms

Inflammatory processes have been implicated in the pathogenesis of ovarian cancer [114]. Prostaglandins play a role in the inflammatory process and their levels are increased during inflammation [115, 116]. Cyclo-oxygenase (COX), also known as prostaglandin G/H synthase, catalyses the conversion of arachidonic acid to prostaglandins and thromboxane [27, 115, 116]. There are two isoforms of cyclo-oxygenase, COX-1 and COX-2 [27, 116]. COX-1 is involved in homeostasis and its levels are usually stable, whereas COX-2 is inducible and its levels rise when tissues are inflamed [115, 116]. NSAIDs act by inhibiting the action of COX-2 and, to some extent COX-1, thus decreasing the production of prostaglandins that mediate inflammation [115, 116]. NSAIDs are commonly used as analgesic, antipyretic, anti-inflammatory or anti-platelet agents; however, paracetamol has little anti-inflammatory effect and has no effect on the function of platelets. This is because it inhibits prostaglandin synthesis in the brain, but has minimal effects on peripheral production. For this reason it is not usually classified as an NSAID [115].

The proposed mechanisms by which NSAIDs decrease the risk of ovarian cancer are related to inhibition of COX [110]. Expression of COX-2 in ovarian epithelial tumours has been demonstrated [117]. COX-2 promotes carcinogenesis by inhibiting apoptosis and promoting metastases. In addition PGE_2, produced via a process catalysed by COX, enhances the proliferation of cells and promotes angiogenesis by enhancing the production of vascular endothelial growth factor (VEGF). Furthermore, PGE_2 promotes the action of aromatase which may lead to increased oestrogen production; oestrogen enhances cell proliferation [27]. Other mechanisms maybe responsible for the inverse association with use of paracetamol. This is because, as noted above, paracetamol has minimal anti-inflammatory effects and weak peripheral inhibition of COX [108].

Paracetamol may confer protection against ovarian cancer via an anti-gonadotrophic effect [108, 110, 113] and depletion of glutathione [108, 110]. Paracetamol has structural similarities with oestrogen and progesterone, hence may exert a negative feedback effect on

the pituitary gland leading to decreased production of gonadotrophins [108]. In addition, enzymatic degradation of paracetamol in the liver involves use of glutathione. Glutathione is essential in the secretion of FSH; its depletion may lead to decreased FSH levels [108].

NSAIDs have also been shown to cause anovulation [118], which may contribute to the observed decrease in the risk of ovarian cancer [110]. Anovulation in this instance is thought to occur as a result of decreased production of LH and prostaglandins resulting from prolonged use of NSAIDs. Prostaglandins are essential in the ovulatory process (rupture of follicle) and LH is responsible for triggering ovulation and for the luteinisation of granulosa cells. However, the amount of LH needed to initiate the ovulatory process is higher than what is needed for luteinisation. In three case reports of women with ovulatory infertility, use of NSAIDs for the treatment of chronic inflammatory conditions resulted in the development of luteinised unruptured follicles (LUF)[2]. Discontinuation of use of NSAIDs resulted in resumption of ovulation [118]. This is further supported by a study in which stronger inverse associations were observed in nulliparous women compared to parous women (OR = 0.58; 95% CI = 0.42-0.80, versus OR = 0.98; 95% CI = 0.71-1.35), and in never-users compared to ever-users of oral contraceptives (OR = 0.47; 95% CI = 0.27-0.82, versus OR = 0.81; 95% CI = 0.64-1.04) [109].

The increase in the risk of ovarian cancer reported with use of NSAIDs may be due to surveillance bias. Use of NSAIDs for conditions that require frequent doctor visits may increase the likelihood of diagnosis of ovarian cancer [100]. This is supported by the findings of Rosenberg et al. [113], in which use of NSAIDs was associated with a statistically significant lower risk of metastatic ovarian cancer, whereas no association was observed for non-metastatic ovarian cancer [113], however, this difference may have been due to chance. In addition, women may be using NSAIDs to relieve early symptoms of ovarian cancer, resulting in higher recent use among cases compared to the general population (reverse causation) [100].

Inconsistencies in findings of different studies may be due to differences in definition of ever-use, lack of inclusion of all NSAIDs used [110], and differences in population exposure to factors known to affect risk of ovarian cancer, such as parity and oral contraceptive use [109]. In addition, the indication for use of NSAIDs may have an influence on the risk of ovarian

[2] a LUF is a fully developed dominant ovarian follicle (approximately 3 cm in diameter), that does not ovulate but undergoes luteinisation and produces progesterone.

cancer [111]. Despite the inconsistencies, the balance of currently available evidence is in favour of an inverse association because this has been found in the most robust study designs (such as prospective cohort studies).

2.5.5 ENDOMETRIOSIS

Endometriosis, first identified and described by Austrian pathologist Carl von Rokitansky [119], is defined as the presence of tissue resembling endometrial glands and stroma outside of the uterus, usually on the surface of the ovary and pelvic peritoneum [119-121], but has also been found in distant sites such as the pleura, pericardium, and brain [119, 120]. The prevalence of endometriosis in women of reproductive age is 5-10% [121, 122] and is higher in women with infertility or pelvic pain [119, 121].

The aetiology of endometriosis has not yet been established [119-121]; several hypotheses have been proposed. These include:

- retrograde menstruation supported by higher risk with cryptomenorrhoea and lower risk with tubal ligation, hysterectomy, and amenorrhoea;
- coelomic metaplasia, probably induced by environmental factors such as radiation and oestrogen [119, 120];
- dissemination by vascular or lymphatic systems; and
- embryonic Müllerian remnants [121].

However, there is a general consensus that the aetiology of endometriosis is multifactorial and includes altered immunity, genetic influences, and hormonal factors [120, 121].

Although endometriosis is a benign condition, it has characteristics similar to those of malignancies such as:

- the potential to metastasize;
- progressive and invasive growth;
- recurrence after treatment;
- and oestrogen-dependent growth [120, 121].

Endometriosis is diagnosed by direct visualisation either at laparotomy or laparoscopy [119]. The goals of treatment for endometriosis are to treat infertility and alleviate pain [119].

Endometriosis has been linked to a higher risk of cancer, with women with a history of endometriosis having an up to 5-fold greater risk of ovarian cancer, 6-fold greater risk of

breast cancer, and 2.5-fold greater risk of non-Hodgkin lymphoma, compared to women with no history of endometriosis [123]. Malignant transformation of endometriosis was first described by Sampson in 1925 [24, 124]; he developed criteria for determining whether a malignancy arises from endometriosis, which were later modified by Scott in 1953. They are known as the Scott and Sampson criteria[3] [120].

Endometriosis and epithelial ovarian tumours have common risk factors [120]. Tubal ligation, hysterectomy, parity, breastfeeding, and oral contraceptive use lower the risk of both ovarian cancer and endometriosis, and infertility increases the risk of both [120, 123], although there may be reverse causation, because endometriosis may cause infertility. Despite this, endometriosis is considered an independent risk factor for ovarian cancer [119, 120]. Endometriosis mainly occurs in women of reproductive age whereas ovarian cancer incidence is highest after menopause; this may be due to the time required from endometriosis to the development of ovarian cancer [120]. Ovarian cancer develops in 5-10% of women diagnosed with endometriosis compared to 1.5% lifetime risk of ovarian cancer in the general population [123]. Endometrioid and clear cell carcinoma are the most common endometriosis-associated ovarian cancer (EAOC) representing 60-80% of EAOC [120]. EAOC is diagnosed at an early stage and is usually of low grade and in younger women and has a better prognosis than other ovarian cancers [120].

In a Swedish prospective cohort study, endometriosis was associated with an overall increase in the risk of ovarian cancer (SIR = 1.2; 95% CI = 1.1-1.3). The risk of ovarian cancer was higher for participants followed up for ≥10 years (SIR = 2.5; 95% CI = 1.4-4.1) [125]. Furthermore, in a study in which women with oophorectomy were excluded, endometriosis was associated with a higher risk of ovarian cancer (SIR = 1.37; 95% CI = 1.14-1.62) and there was no statistically significant difference in risk between parous and nulliparous women [74]. However, findings of these studies may represent moderate-to-severe endometriosis cases because study subjects were hospitalised with that diagnosis [74, 125].

Similarly, a population-based case-control study in Los Angeles County reported a statistically significant higher risk of ovarian cancer in women with a history of

[3] Scott and Sampson criteria: (i) Demonstration of clear evidence of endometriosis in proximity to the tumour; (ii) the tumour must be seen to arise in endometriosis rather than invade from another site; (iii) the histological appearance should be that of endometriosis with demonstration of both glands and stroma. Scott added a 4th criterion, namely the histological demonstration of transition from benign endometriosis to cancer within the endometriosis.

endometriosis (RR = 1.95; 95% CI = 1.20-3.17). Recent diagnosis of endometriosis (2-10 years) was associated with higher risk of ovarian cancer (RR = 2.58) than diagnosis more than 10 years previously (RR = 1.58); however, the finding of higher risk of ovarian cancer more than 10 years after diagnosis means that screening bias cannot completely explain the association [100].

Risk of ovarian cancer associated with endometriosis has been shown to differ by histologic type, with greatest risk seen for endometrioid and clear cell tumours [100, 101]. In a nationwide population-based study in Australia, overall, there was a statistically non-significant association (OR = 1.31; 95% CI = 0.97-1.78). However, a statistically significant higher risk of endometrioid (OR = 1.85; 95% CI = 1.02-3.38) and clear-cell carcinoma was observed (OR = 2.66; 95% CI = 1.31-5.44) [101].

The prevalence of EAOC may be underestimated because endometriosis may be asymptomatic and thus remain undiagnosed [125]. Diagnosis requires direct visualisation [119], which may be influenced by the standard of medical care available to the study population [125]. Some studies do not indicate whether the Sampson and Scott criteria were applied, which may contribute to variation in study findings [120]. Strict adherence to these criteria may not always be possible because they require extensive sampling and/or sectioning, and aggressive tumours may destroy all endometriotic tissue, making it difficult to meet the criterion of finding benign endometriosis close to malignant tissue [119, 120]. Without strict application of the criteria, the prevalence of EAOC has been found to be higher [120]. In comparing findings from different studies, effects of different treatment modalities for endometriosis and variation in these over time should be considered. These treatments include oophorectomy, progesterones, danazol, oral contraceptives, and letrozole [123].

2.5.5.1 Possible mechanisms

It is not clear whether the association between endometriosis and ovarian cancer is a result of shared risk factors or a direct progression from endometriosis to cancer [119, 122]. There have been suggestions that genetic defects may be present in benign endometriosis which allow malignant transformation, prossibly through an intermediate lesion that is yet to be identified [121]. Similar genetic alterations (loss of heterozygosity) have been observed in malignant tissue and adjacent ovarian cancer, which supports the notion of ovarian cancer developing from endometriosis [120]. In addition, progression from benign epithelium

(endometriosis) to atypical endometriosis followed by carcinoma has been reported in case series [120].

Several aetiologic pathways for EAOC have been proposed. High local oestrogen concentrations in endometriotic lesions may be responsible for the higher risk of ovarian cancer [121, 123]. Oestrogen promotes cell proliferation which may lead to malignant transformation [121, 123, 124]. Endometriotic tissues have high levels of aromatase, an enzyme that converts androgens to oestrogens [121, 123]. In addition, endometriotic tissue lacks 17-β-hydroxy steroid dehydrogenase (17-β-HSD) type 2, an enzyme that converts oestradiol into the less potent oestrone [121, 123, 124], while it has normal levels of 17-β-HSD type 1, which converts oestrone to oestradiol [121, 123]. The net effect is an increase in the levels of the more potent oestradiol due to an increase in production and a decrease in inactivation [121]. Eutopic endometrium produces normal levels of 17-β-HSD types 1 and 2 and lacks aromatase [121, 123]. In addition, oestradiol stimulates the increased production of $PGE_{2,}$ which, in turn, activates the aromatase enzyme, resulting in a positive feedback loop. PGE_2 also stimulates proliferation of cells [121, 123, 124]. Due to the increased conversion of androgens to oestrogens in endometriotic lesions, increased levels of androgens are expected to promote the growth of endometriosis. Despite this, synthetic androgens are used to treat endometriosis. This inconsistency may be explained by the fact that synthetic androgens inhibit the release of gonadotropins, leading to a hypo-oestrogenic state [123, 126].

DNA damage may also occur as a result of oxidative stress. Accumulation of heme and iron in endometriotic cysts as a result of repetitive haemorrhage may result in excess production of reactive oxygen species (ROS), which, in turn, may lead to DNA mutation [121, 122].

There is alteration of cellular and humoral immunity in endometriosis, which favours growth of endometriotic lesions [23, 123, 126]. This, in turn, may promote malignant transformation and tumour growth [123, 126]. Peritoneal fluid surrounding endometriotic lesions has increased concentrations of inflammatory cells (including natural killer [NK] cells), however, they are functionally deficient [23, 123, 126]. In addition, peritoneal macrophages produce matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF), both of which promote angiogenesis and the invasiveness and spread of both endometriosis and tumours. Furthermore, cytokines produced by inflammatory cells stimulate increased production of PGE_2, which increases cell proliferation, and impairs apoptosis [123, 126]. All

these events act to sustain and cause progression of endometriosis and may favour malignant transformation of endometriotic foci/lesions and growth of the resulting tumours [123, 126]. Endometriosis has also been linked to autoimmune conditions [23, 123, 126]: higher rates of systemic lupus erythematosus (SLE), Sjögrens syndrome, and thyroiditis have been reported [123, 126].

2.5.6 GYNAECOLOGIC SURGERY

Tubal ligation and hysterectomy have been shown to be inversely associated with ovarian cancer [7, 103]. A meta-analysis found a 34% lower risk of epithelial ovarian cancer in women with history of tubal ligation (RR = 0.66; 95% CI = 0.60-0.70). A stronger inverse association was observed for invasive ovarian tumours (RR = 0.68; 95% CI = 0.61-0.75) than for borderline tumours (RR = 0.86; 95% CI = 0.67-1.10). The inverse association varied according to the histological type of the tumour: among women with invasive ovarian cancer, a statistically significant lower risk was observed in those with endometrioid (RR = 0.40; 95% CI = 0.30-0.53) and serous tumours (RR = 0.73; 95% CI = 0.63-0.85), but not mucinous tumours (P = 0.653). The inverse association was apparent 10-14 years after tubal ligation (RR = 0.65; 95% CI = 0.44-0.97; P = 0.034). There was no relationship with age at tubal ligation [103].

Findings consistent with those of the above study were reported in a pooled analysis of 13 population-based case-control studies. A 29% lower overall risk of invasive ovarian cancer was observed in women with a history of tubal ligation (OR = 0.71; 95% CI = 0.66-0.77; P<0.001), but there was no association between tubal ligation and the risk of borderline ovarian tumours (0.98; 95% CI = 0.86-1.12; P = 0.80). The strength of the inverse association was significantly different among the histological subtypes of invasive ovarian cancer (P<0.001): compared with unsterilized women, tubal ligation was most strongly inversely associated with endometrioid (OR = 0.48; 95% CI = 0.40-0.59; P<0.001) and clear-cell tumours (OR = 0.52; 95% CI = 0.40-0.67; P<0.001), followed by mucinous tumours (OR = 0.68; 95% CI = 0.52-0.89; P = 0.005), and was more weakly associated with serous tumours (OR = 0.81; 95% CI = 0.74-0.89; P<0.001). The inverse association was evident >30 years after tubal ligation, with an OR for invasive cancer of 0.72 (95% CI = 0.61-0.84). Tubal ligation at any age was associated with a lower risk of ovarian cancer. The magnitude of the inverse association with tubal ligation did not differ by time since tubal ligation, stage or grade of the tumour, and was not affected by hysterectomy status [59].

Studies point to an inverse association even in women with *BRCA1* and *2* mutations. In the above meta-analysis, a statistically significant lower risk was observed in *BRCA1*-mutation carriers (RR = 0.69; 95% CI = 0.53-0.89; P = 0.004) but no statistically significant inverse association was found in *BRCA2*-mutation carriers [103]. In addition, in a case-control study of women with *BRCA1/2* mutations, tubal ligation was associated with a lower risk of ovarian cancer in *BRCA1*-mutation carriers (OR = 0.39; 95% CI = 0.22-0.70; P = 0.002). Lower risk was not observed in *BRCA2*-mutation carriers (OR = 1.19; 95% CI = 0.38-3.68); however, power was low for this group. Among *BRCA1*-mutation carriers, the strength of association with tubal ligation was greater when the procedure was done at a younger age (OR = 0.36; 95% CI = 0.15-0.94, for TL at < 30 years, OR= 0.38; 95% CI = 0.20-0.73 for TL at 30-39, and OR = 0.46; 95% CI = 0.18-2.11 for TL at 40-51 years), although there was no statistically significant trend (P-trend = 0.11). Use of oral contraceptives was associated with a statistically significant lower risk of ovarian cancer (OR 0.44; 95% CI = 0.28-0.68). The inverse association was statistically significant for carriers of both *BRCA1* (OR = 0.48; 95% CI = 0.29-0.80) and *BRCA2* mutations (OR = 0.35; 95% CI = 0.15-0.83). Among *BRCA1*-mutation carriers, compared to women who had never used OCs and were not sterilised, TL combined with ever-use of OCs had a stronger inverse association than each method alone (OR = 0.28; 95% CI = 0.15-0.52; P <0.001) [104].

Hysterectomy has been shown to be inversely associated with ovarian cancer, with studies reporting a 30-50% lower risk [63]. In a cohort study, a history of hysterectomy was associated with an HR of 0.50 (95% CI = 0.34-0.72) [127]. In a population-based case-control study, the inverse association in women with a history of both tubal ligation and hysterectomy was stronger than in those with history of either operation alone (TL alone: OR = 0.5; 95% CI = 0.4-0.7, hysterectomy alone OR = 0.8; 95% CI = 0.6-1.1, and for both operations: OR = 0.4; 95% CI = 0.2-0.8). There was no difference in the risk of ovarian cancer between women who had had a vaginal hysterectomy (OR = 0.6; 95% CI = 0.3-1.0) and those who had had an abdominal hysterectomy (OR = 0.9; 95% CI = 0.6-1.3). History of laparoscopy for reasons other than ovarian conditions or tubal ligation was associated with an OR of 1.0 (95% CI = 0.7-1.5). There was no relationship between risk of ovarian cancer and duration since hysterectomy [87].

2.5.6.1 Possible mechanisms

Several mechanisms have been proposed to explain the relationship between tubal ligation and ovarian cancer. The inverse association may be due to selection or screening bias. Tubal

ligation, being a permanent contraceptive method, is usually done at an older age after the completion of childbearing; this may translate to longer duration of use of oral contraceptives and higher parity which may be responsible for the observed risk reduction [103]. However, in studies in which the risk estimates were adjusted for parity and oral contraceptive use [59, 104] an inverse association was still observed. In addition, no clear relationship with age at tubal ligation has been observed [59, 103, 104]. Abnormal or suspicious ovaries may be removed during tubal ligation or hysterectomy resulting in surveillance bias. If this were true, a diminishing trend across the inverse association would be observed [128]. Time since tubal ligation has not been shown to affect the magnitude of the inverse association against ovarian cancer accorded by tubal ligation [59, 103, 128]. In addition, abdominal hysterectomy, which allows for inspection of the ovaries during the operation is not more strongly inversely associated with risk than vaginal hysterectomy with no inspection possible, and laparoscopy for reasons other than tubal ligation and ovarian conditions, which also allows for the inspection of the ovaries, is not inversely associated with risk [87].

Tubal ligation and hysterectomy may block substances that may be carcinogenic from reaching the peritoneal cavity/ovary from the vagina and perineum [87, 103, 104, 127, 128]. However, findings regarding the relationship between the risk of ovarian cancer and infectious agents or talc have been inconsistent [103, 128]. In addition, the increase in risk of ovarian cancer associated with use of talc is low, and its elimination does not account for the inverse association observed with tubal ligation [104].

It has also been proposed that the inverse association of tubal ligation with ovarian cancer risk and the observed differences in the strength of that association with different subtypes of epithelial ovarian tumours is due to their different cells of origin and the extent to which gynaecological surgery or tubal ligation eliminates or prevents these cells from reaching the ovary [59, 103]. The smaller inverse association observed for serous tumours may be because the tubal cells are not completely eliminated [59]. This theory also serves to explain situations in which null findings are observed in the relationship between ovarian cancer and tubal ligation. A statistically significant lower risk of ovarian cancer after tubal ligation may not be observed because serous tumours, which form the bulk of epithelial ovarian tumours, are thought to arise from the distal tubal epithelium, which is not routinely removed during tubal ligation or hysterectomy [129, 130]. In view of the above observations, salpingectomy which eliminates cells from distal tubes may show a stronger inverse association with ovarian cancer [59, 129]. Mucinous tumours are thought to arise from embryonic remnants of

transitional-type epithelial cells located at the tubal-peritoneal junction. This, in addition to the observation that mucinous tumours seem to have a different risk-factor profile from that of other epithelial ovarian tumours may explain the minimal-to-no inverse association observed with tubal ligation [59]. The observation that the strength of the inverse association between ovarian cancer and tubal ligation is not affected by duration since tubal ligation or age at tubal ligation [59, 103, 104] lends further credence to this hypothesis. Gynaecological surgery permanently eliminates or obstructs potential cancer cells from reaching the ovaries, resulting in a permanent risk reduction [59].

It has been hypothesised that tubal ligation and/or hysterectomy leads to compromise of blood supply to the ovaries leading to changes in ovarian function. This leads to alteration in hormone levels and abnormal uterine bleeding [59, 104, 127, 128]. However, findings regarding changes in hormonal levels and alterations in menstrual patterns have been inconsistent [128].

The inverse association with gynaecological surgery is also thought to result from an immune response with increased production of antibodies against mucin [59, 86]. In a population-based case-control study, factors that promote expression of anti-MUC1 were associated with a lower risk of ovarian cancer. Among the controls, use of oral contraceptives and history of pelvic surgery (tubal ligation, cervical conisation, and caesarean sections) were associated with a statistically significantly higher probability of having antibodies compared to the unexposed (40.7% vs 26.7%; P = 0.05, and 47.2% vs 30.9%; P = 0.01, respectively). These antibodies or some related immune response may then be responsible for the observed lower risk of ovarian cancer [86].

2.5.7 EXOGENOUS HORMONES

2.5.7.1 Hormonal contraceptives

Use of oral contraceptives has been consistently associated with a lower risk of ovarian cancer. The association with use of other types of contraceptives is not yet clear. The association between contraceptive use and the risk of ovarian cancer will be discussed in detail in Chapter 4.

2.5.7.2 Post-menopausal hormone

Post-menopausal hormone (PMH) use (earlier called hormone replacement therapy [HRT]) was first introduced in the 1940s for the treatment of postmenopausal symptoms, and was oestrogen-based. An increase in the risk of endometrial cancer was observed, and in the

1980s, progesterone was added to counter this effect [131]. The use of PMH increased when it was shown in observational studies to confer protection against cardiovascular disease, dementia, loss of bone mineral density, and osteoporosis. The findings of Women's Health Initiative (WHI) [132], a large randomised clinical trial, in 2002, that PMH does not decrease the risk of cardiovascular disease and may, instead, increase the risk of both cancer and cardiovascular disease, led to a widespread decline in the use of PMH [131, 133]. Since its introduction, in order to improve safety, the oestrogen dose has been reduced greatly. Use of PMH for the treatment of postmenopausal symptoms may be beneficial for women below 60 years of age, whereas the risks associated with PMH use are high in women over 70 [131]. PMH can be administered as oestrogen alone (in women who have had a hysterectomy) or in combination with progestins and via different routes [131].

Use of PMH has been shown to increase the risk of endometrial and breast cancer [134]. It is becoming increasingly clear that PMH increases the risk of ovarian cancer [135]. A positive association between PMH use and the occurrence of ovarian cancer has been reported in many studies [134-141]. In a systematic review of 52 epidemiological studies, PMH use was associated with a higher risk of ovarian cancer (RR = 1.4; 95% CI = 1.1-1.9; P <0.001) [138]. Higher risk has been observed in long-term, compared to short-term, users [133, 135, 136, 141]. A substantial higher risk was observed with long duration (≥10 years) of use of PMH (oestrogen only: RR = 2.15; 95% CI = 1.30-3.57, and oestrogen plus progestin: RR = 1.68; 95% CI = 1.13-2.49) compared to use for <10 years (oestrogen only: RR = 1.25; 95% CI = 0.71-2.20, and oestrogen plus progestin: RR = 1.33; 95% CI = 0.98-1.79) compared with never-use [141]. A null association with duration of use has also been observed [134]; however, in this study the majority of participants had used PMH for <5 years and assessment of association with use for >10 years was not done. The risk associated with PMH has been shown to decrease with time since discontinuation of use [133, 136, 138, 140]: in a study done across 10 European countries (European Prospective Investigation into Cancer and Nutrition), compared with never-use, use of PMH was associated with an HR of 1.29 (95% CI = 1.01-1.65) for current users and an HR of 1.16 (95% CI = 0.94-1.43) for ever-users, and no association for past users (HR = 0.96; 95% CI = 0.70-1.30) [136]. In the previously mentioned systematic review, risk diminished with longer duration since last use, but was apparent for >5 years since last use in women who had used PMH for ≥5 years (RR = 1.10; 95% CI = 1.01-1.20; P = 0.02) [138].

Several studies have reported lower risk of ovarian cancer with use of oestrogen-progestin (EPT) preparations compared to oestrogen-only preparations (ET) [133, 135-137]. In a US prospective cohort study of 54,436 post-menopausal women, compared to never-use, ever-use of ET (may have included women who had not had a hysterectomy) was associated with a statistically significant higher risk of epithelial ovarian cancer (RR = 2.07; 95% CI = 1.50-2.85) with a clear trend of higher risk with longer duration of use (P-trend = 0.01). Each additional 5 years of use was associated with an RR of 1.25 (95% CI = 1.15-1.36). No association with risk was observed in former ET users. Among EPT users, there was no association with the risk of ovarian cancer among current and past users [137]. Consistent with this, a meta-analysis of 14 population-based studies (8 population-based case-control studies, 5 cohort studies, and 1 RCT), reported a statistically significant difference in the risk of ovarian cancer between ET and EPT users (P = 0.004). For every 5 years of use, a 22% higher risk was observed in ET users (RR = 1.22; 95% CI = 1.18-1.27), compared to a 10% higher risk in EPT users (RR = 1.10; 95% CI = 1.04-1.16; P = 0.001) [135] although some studies have observed no differences in risk according to type of PMH used [138, 141]. Mixed results have been observed in relation to use of sequential or continuous progestins; no differences in risk estimates between sequential and continuous use [141], and lack of association with sequential use but an inverse association with continuous use have been reported [139].

Use of PMH has been associated with a higher risk for endometrioid and serous tumours compared to other histological types [138, 139]. A statistically significant difference in the risks associated with PMH use between the histological subtypes of epithelial ovarian tumours (P <0.001) has been reported, with the greatest risk seen in serous tumours (RR = 1.42; 95% CI = 1.40-1.66; P <0.001) and endometrioid tumours (RR = 1.42; 95% CI = 1.20-1.67; P <0.001) [138]. In addition, in a prospective cohort study, use of oestrogen-only preparations (ET) was associated with a statistically significant higher risk of serous (IRR = 1.7; 95% CI = 1.4-2.1) but not endometrioid tumours (IRR = 1.4; 95% CI = 0.9-2.1), and was inversely associated with mucinous tumours (IRR = 0.3; 95% CI = 0.1-0.8) and had no association with clear-cell tumours (IRR = 0.6; 95% CI = 0.2-1.5). Similar findings were observed with use of oestrogen-plus-progestin preparations, except that no association was observed with mucinous tumours. A clear trend of higher risk with longer duration of use was observed for serous tumours, but not for other histological subtypes [139].

In the UK Million Women Study, use of PMH was associated with an increase in the incidence of ovarian cancer and mortality from ovarian cancer. Relative to never-use, current use was associated with an RR of 1.20 (95% CI = 1.09-1.32) for incidence of ovarian cancer, and RR = 1.23 (95% CI = 1.09-1.38) for mortality from ovarian cancer. No association was observed between past use and the incidence of ovarian cancer or mortality from ovarian cancer. Risk did not differ by type of PMH used or mode of administration. A statistically significant trend toward higher risk with longer duration of use was observed (P = 0.04). It was estimated that, over a period of 5 years, use of PMH in the UK had led to the development of 1 extra ovarian cancer case per 2,500 users, and 1 extra death from ovarian cancer per 3,300 users, which is equivalent to 1,300 extra ovarian cancer cases and 1,000 extra deaths [142]. However, in a nationwide prospective cohort study of 799 women diagnosed with epithelial ovarian cancer (both borderline and invasive) in Sweden, no statistically significant difference in survival from epithelial ovarian cancer was observed between users and non-users of PMH (HR = 0.83; 95% CI = 0.65-1.08) and this did not vary by type of PMH used. No difference was observed by histological type [143].

2.5.7.2.1 Possible mechanisms

PMH use may have an aetiological association with ovarian cancer. This is due to the observed increase in incidence of ovarian cancer in current users, diminished risk after discontinuation of use, and the stronger association with risk of serous and endometrioid tumours [138]; thus meeting the Bradford Hill criteria of strength, temporality, and specificity [144]. Use of ET in postmenopausal women should decrease levels of gonadotropins, a hormone thought to increase the risk of ovarian cancer. However, it seems that the effect of excess oestrogen on the occurrence of ovarian cancer overrides its suppressive effects on gonadotropin production [133]. Oestrogens enhance the proliferation of ovarian epithelial cells and therefore increase the risk of ovarian cancer, whereas progesterone promotes apoptosis and therefore lowers risk. The lower risk observed with use of oestrogen plus progestin preparations, compared to oestrogen-only preparations suggests that progestins mitigate the effects of oestrogen [135-137].

The differences in risk across the different histological types support the notion of different cells of origin. The observed increase in the risk of endometrioid tumours with the use of PMH (especially ET) is similar to the increase in risk of endometrial cancer. On the other hand, the lower risk of mucinous tumours is similar to the observed association between PMH (particularly ET) and risk of colon cancer [139].

Increase in risk may also be due to surveillance bias because women on PMH require regular doctor visits, which may increase the chance of diagnosis of ovarian cancer. However, this argument is challenged by a study in which tumours were not diagnosed at an early stage for PMH users [139].

In view of the observed increase in incidence of ovarian cancer and mortality from ovarian cancer, this should be considered in any risk-benefit assessment regarding use of PMH.

2.5.8 LIFESTYLE FACTORS

2.5.8.1 Alcohol

Alcohol use is associated with a higher risk of cancer of the oral cavity, pharynx, larynx, oesophagus, large bowel, and breast [145, 146]. Studies regarding the relationship between alcohol consumption and ovarian cancer risk have had mixed results [147, 148]. A pooled analysis of 5 case-control studies showed a dose-response relationship between alcohol intake and the risk of ovarian cancer. Intake of 25g/day was associated with an RR of 1.11 (95% CI = 1.00-1.24); 50g/day RR = 1.23 (95% CI = 1.01-1.54); and 100g/day RR = 1.53 (95% CI = 1.03- 2.32) [146]. Furthermore, risk difference by histologic type [149] and type of alcoholic beverage have been reported [147, 149]. In a population-based case-control study, lifetime alcohol intake was associated with higher risk of mucinous (OR = 1.93; 95% CI = 1.02-3.65; P = 0.04, for heavy drinkers compared to never drinkers) but not non-mucinous ovarian tumours. Risk differed according to type of alcohol with the greatest risk for mucinous tumours seen in users of spirits (adjusted OR = 8.8; 95% CI = 2.9-27.0; P <0.001). Overall, there was no association between alcohol intake and the risk of ovarian cancer (all histological types) [149].

Lack of an association between alcohol intake and the risk of ovarian cancer has been reported in cohort studies (which are generally more robust than population-based case-control studies) [145, 147]. A pooled analysis of 10 prospective cohort studies observed no association between moderate alcohol intake and the risk of invasive EOC (RR = 1.12; 95% CI = 0.86-1.44, for ≥30 versus 0g/day). The risk did not differ by type of alcoholic beverage (wine, beer, spirits) or histological subtype of EOC, and the association was not modified by oral contraceptive use, PMH use, parity, folate intake, menopausal status, BMI, or smoking [145]. Similarly, in a prospective study (California Teachers Study [CTS]), no relationship was seen between the risk of EOC and total alcohol intake (RR = 1.08; 95% CI = 0.79-1.48 vs non-drinkers) or intake of beer or liquor only (RR = 1.03; 95% CI = 0.58-1.83 vs non-

drinkers). In contrast, compared to non-drinkers, women who drank wine had a higher risk of ovarian cancer (RR = 1.40; 95% CI = 1.01-1.93); the risk associated with wine intake was not altered by adjusting for race, daily energy intake, physical activity, parity, age, or menopausal status (multivariate RR = 1.70; 95% CI = 1.10-2.62). Adjustment for folate intake and determinants of alcohol intake in the study did not alter risk. Use of oestrogen-based PMH for >5 years in combination with wine intake (in the year before interview) was associated with a higher risk of ovarian cancer (RR = 2.39; 95% CI = 0.97-5.89; P-trend = 0.02). However, no association was observed in women who used oestrogen-progestin PMH (RR = 1.34; 95% CI = 0.51-3.54, P-trend = 0.29) [147].

2.5.8.1.1 Possible mechanisms

Alcohol may have both direct and inverse associations with ovarian cancer [148]. The exact mechanism by which alcohol may affect the risk of ovarian cancer is not known; several possible mechanisms have been proposed [145, 147, 149]. Proposed mechanisms associated with a possible lower risk include: decreased gonadotropin (LH and FSH) levels [145] and ovulation suppression [148].

Alcohol increases the levels of oestrogen and androgens, which, in turn, may increase the risk of ovarian cancer [145, 147, 149]. The association of alcohol intake and the risk of ovarian cancer in oestrogen-based PMH users supports the notion of increased oestrogen levels playing a role in the increase in risk of ovarian cancer as a result of alcohol consumption [147]. Other mechanisms that may explain an increase in risk include:

- damage to DNA [147] through its first oxidative metabolic product, acetaldehyde [145];
- abnormal folate metabolism [145, 147];
- impaired clearance of carcinogens and promotion of tumour spread [147].

The higher risk in mucinous tumours may be related to their similarity to intestinal epithelial cells: alcohol increases risk of large bowel cancer [149].

Inconsistencies in the association between alcohol use and the risk of ovarian cancer may be due to lack of adjustment for smoking or behaviours associated with drinking in some studies and the lack of standard definition of what constitutes an alcoholic beverage [149] or inconsistencies in determination of dose. Difference in ovarian cancer risk in relation to type of alcoholic beverage may be related to differences in lifestyle factors among users of the

different types of alcohol [149]. In addition, constituents other than alcohol may influence risk of ovarian cancer [147].

2.5.8.2 Smoking

There is no consistent association between cigarette smoking and the overall risk of ovarian cancer. However, several studies have reported a higher risk of mucinous tumours [150]. In a population-based case-control study in Australia, history of smoking was associated with a higher risk of EOC (adjusted OR = 1.5; 95% CI = 1.2-1.9). The observed risk was higher for borderline tumours (OR = 2.4; 95% CI = 1.4-4.1) than for invasive tumours (OR = 1.7; 95% CI = 1.2-2.4). The strongest relationship was observed with mucinous tumours: the OR for current smokers was 3.2 (95% CI = 1.8-5.7) and that for past smokers was 2.3 (95% CI = 1.3-3.9). A higher risk was observed in current than past smokers; however, no statistically significant trend was observed with greater pack years. There was no relationship with age at initiation of smoking or duration of smoking [150].

Consistent with the above study are the findings of a pooled analysis of 51 epidemiologic studies (published and unpublished), in which smoking was weakly associated with a higher risk of ovarian cancer (RR = 1.06; 95% CI = 1.03-1.09) and there was no difference in risk between current and past smokers. A higher risk of mucinous tumours was seen among current smokers than among never-smokers (RR = 1.79; 95% CI = 1.60-2.00; P <0.001), whereas an inverse association was seen for endometrioid tumours (RR = 0.81; 95% CI = 0.72-0.92). A statistically significant difference in risk was observed across the histological subtypes of epithelial ovarian tumours (P-heterogeneity <0.001): the positive association seen with mucinous tumours was higher for borderline tumours than for invasive tumours (RR = 2.25; 95% CI = 1.91-2.65, and RR = 1.49; 95% CI = 1.28-1.73, respectively; P-heterogeneity = 0.01) [151]. Purdie et al. also reported a higher risk of mucinous than non-mucinous tumours in ever-smokers (OR = 2.65; 95% CI = 1.66-4.22 versus OR = 1.36; 95% CI = 1.07-1.74; P-difference = 0.001). In contrast, there was no relationship between smoking and risk of endometrioid tumours (OR = 0.95; 95% CI = 0.57-1.60) [152].

Studies have also reported no association between smoking and the overall risk of ovarian cancer, but a higher risk specifically of mucinous tumours. In a population-based case-control study in the US (part of the Cancer and Steroid Hormone Study), smoking was associated with risk of mucinous tumours (OR = 2.3; 95% CI = 1.4-3.9), but not of non-mucinous tumours (OR = 1.0, 0.9, and 0.7 for endometrioid, serous, and other tumours). The higher risk

of mucinous tumours among current smokers was not altered by duration of smoking or age at initiation of smoking. A statistically non-significant trend was observed with more pack years among current smokers, with ORs adjusted for OC use and parity [153]. Similarly, in a case-control study, smoking was associated with a higher risk of mucinous tumours (OR = 1.9; 95% CI = 1.3-2.9), but not non-mucinous tumours (OR = 1.1; 95% CI = 0.9-1.3). The risk of mucinous tumours was even higher among current smokers (OR = 2.7; 95% CI = 1.7-4.3), but not elevated in non-mucinous tumours. The association with mucinous tumours was not affected by duration or age at initiation of smoking, but there was a statistically significant trend toward higher risk with more pack years (P-trend = 0.01) [149].

2.5.8.2.1 Possible mechanisms

Smoking has been shown to cause a higher risk of cancer in tissues distant from the immediately inhaled smoke: cervix, bladder, kidney, and pancreas [150]. In addition, smoking has been shown to cause DNA damage in epithelial cells and nicotine has been found in cervical epithelial cells [150, 154]. Furthermore, cigarette metabolites have been found in granulosa cells of active and passive smokers [149, 154]. Cigarette smoking has also been shown to affect ovarian function. An earlier age at menopause has been reported in general [155] and also in *BRCA1/2* mutation carriers: *BRCA1/2* mutation carriers who were current smokers had menopause at an earlier age relative to non-smokers (46 years versus 49-50 years) [156]. This may be due to accumulation of cigarette metabolites in the ovary [156].

The different associations between smoking and different histological types of ovarian cancer may be explained by difference in the cells of origin. Smoking has been shown to increase the risk of cervical and a subset of colon cancers. Mucinous tumours contain mucin-producing epithelial cells that resemble epithelia of the cervix and intestines [149, 154]. The inverse association observed with endometrioid tumours is similar to that of endometrial cancer [152, 154], which may be explained by the histological similarity between the two tumours [150, 152]. This may be due to a decline in oestrogen associated with smoking [150, 154], and the inverse relationship between BMI and smoking [154] – which can induce earlier menopause [155, 156]. These histological similarities may make this association biologically more credible [149, 154].

2.5.9 ANTHROPOMETRIC MEASURES

2.5.9.1 Height

Height has been associated with higher risk of breast cancer [157, 158]. Studies assessing the association between height and the risk of ovarian cancer have either reported no association

or a positive association. In a pooled analysis of 12 prospective cohort studies, for every 5cm greater height, a 10% higher risk of ovarian cancer was observed (RR = 1.10; 95% CI = 1.05-1.15). This association may have been stronger in premenopausal women than in postmenopausal women: relative risks of 1.20 (95% CI = 1.05-1.37) and 1.07 (95% CI = 1.02-1.13) were observed for every 5cm increase in height in pre- and post-menopausal women respectively (P-interaction = 0.14) [159]. Similarly, in a prospective cohort study of postmenopausal women (Netherlands Cohort Study on Diet and Cancer), taller women were at a greater risk of ovarian cancer than women with a height of ≤160 cm: women who were >175 cm tall had a rate ratio (RR) of 2.17 (95% CI = 1.14-4.13; P-trend = 0.01) [158]. On the other hand, in a hospital-based case control study done in New York, there was no association between risk of ovarian cancer and height. Only a slight statistically non-significant higher risk was observed with height in premenopausal women (P = 0.298) [160]. However, cohort studies are less vulnerable to bias than hospital-based case-control studies.

Height has been shown to be associated with risk of mortality from ovarian cancer. A prospective cohort study of 300,537 post-menopausal women in the US, reported that greater height was associated with higher risk of mortality from ovarian cancer (P-trend = 0.01). Women who were ≥177 cm tall had an RR of 1.41 (95% CI = 0.95-2.09) compared to women who were 152-156 cm tall [161].

2.5.9.2 BMI

Obesity has consistently been associated with a higher risk of cancer of the breast, endometrium, gallbladder, bowel, kidney, and pancreas [159]. Findings regarding the association between BMI and ovarian cancer have not been consistent [159, 161, 162]. The majority of studies have demonstrated a higher risk in women who were obese for most of their adult life [158, 160, 163-165]. In a nationwide population-based case-control study in Israel, relative to women whose BMI was in the first quartile, those in the fourth quartile at 18 years and most of adult life, had a statistically significant higher risk of ovarian cancer (OR = 1.42; 95% CI = 1.12-1.89; P-trend = 0.009 and OR = 1.51; 95% CI = 1.20-1.91; P-trend = 0.001, respectively). These findings did not vary by tumour invasiveness (borderline or invasive), or *BRCA* carrier status. Change in BMI between 18 years and most of adult life was not associated with risk of ovarian cancer (P-trend = 0.26) [164]. A population-based case-control study in the USA found, relative to women who had normal weight throughout adulthood (18 years, 30 years and 5 years before reference date), obesity 5 years before reference date was associated with an RR of 1.6 (95% CI = 0.9-3.1), obesity at 30 years of

age and 5 years before the reference date was associated with an RR of 1.5 (95% CI = 0.7-3.0), and obesity throughout adulthood was associated with a RR of 1.4 (95% CI = 0.5-3.8) [165]. None of these findings was statistically significant. Lack of an association between adult BMI and the risk of ovarian cancer has also been reported in other studies [157, 159].

BMI at other stages of life and change in BMI in adulthood have not been consistently associated with the occurrence of ovarian cancer. A pooled analysis of 11 population-based case-control studies reported a statistically significant higher risk of ovarian cancer in obese women (OR = 1.4; 95% CI = 1.2-1.6). A trend toward higher risk with higher BMI was observed (OR = 1.03; 95% CI = 1.01-1.06; for each unit increase in BMI). These findings were not changed by adjusting for energy and fat intake. A statistically significant higher risk was observed in women who gained ≥30kg in adulthood relative to those who gained <5kg (OR = 1.5; 95% CI = 1.0-2.3). There was no association with BMI at 20 years of age [166]. In contrast, a large prospective cohort study of about 1.1 million Norwegian women reported a statistically significant positive association between BMI at the age of 14-19 years and the risk of invasive epithelial ovarian cancer: compared to girls with a medium BMI (25^{th}-74^{th} percentile), high (75^{th}-84^{th} percentile) and very high (≥85^{th} percentile) BMI were associated with a higher risk of ovarian cancer (RR = 1.43; 95% CI = 1.00-2.04, and RR = 1.56; 95% CI = 1.04-2.32, respectively). No association was observed with adult BMI [157]. Leitzman et al. observed no association between weight gain from 18 years of age and the risk of ovarian cancer [163].

A more obvious higher risk has been observed in never-users compared to ever-users of PMH [163] and in premenopausal compared to postmenopausal women [159, 160]: in a prospective cohort study in the US that included 94,525 US women (638,510 person years of follow-up), obesity was associated with a statistically non-significant higher risk of ovarian cancer (RR = 1.26; 95% CI = 0.94-1.68, for BMI ≥30kg/m^2 versus 18.5-24.9kg/m^2 [normal BMI]). There was a suggestion of trend toward higher risk with higher BMI. Compared to a BMI of 18.5-24.9kg/m^2, a BMI of >35kg/m^2 was associated with an RR of 1.38 (95% CI=0.92-2.09). Higher risk was observed in women who had never-used PMH, but not in ever-users (obesity versus normal weight: RR = 1.83; 95% CI = 1.18-2.84, and RR = 0.96; 95% CI=0.65-1.43, P-interaction = 0.02). The effect modification by PMH use did not differ by type of PMH used or hysterectomy status. In an analysis confined to women who had never used PMH, no statistically significant association with mortality was observed in obese women relative to

normal weight women (RR=1.51; 95% CI=0.91-2.51). There was no association between weight gain since 18 years of age and the risk of ovarian cancer. In contrast, women who were obese at baseline and also at 18 years of age and had either used PMH or had a family history of ovarian cancer, had a higher risk of ovarian cancer relative to those who had normal weight at both time points (RR = 2.99; 95% CI = 1.69-5.29; P-interaction = 0.002, and RR = 1.68; 95% CI = 1.05-2.68; P-interaction = 0.09, respectively) [163].

A hospital-based case-control study done in New York, reported a statistically significant higher risk of ovarian cancer in obese pre-menopausal women (OR = 2.19; 95% CI = 1.19-4.04), with a clear trend of higher risk with higher BMI (P-trend = 0.021). In contrast, there was no association between obesity and the risk of ovarian cancer in postmenopausal women (OR = 0.88; 95% CI = 0.58-1.33; P-trend = 0.56) [160]. Similarly, in a pooled analysis of 12 prospective cohort studies, BMI at entry into the study had no relationship with the overall risk of ovarian cancer (P-trend = 0.90) or the risk of serous, endometrioid, or mucinous tumours when each was assessed separately. There was also no difference in risk by age (P-difference = 0.73). However, there was the suggestion of a difference in risk between pre- and post-menopausal women (P-interaction = 0.07). An increase in BMI of 4kg/m^2 in premenopausal and postmenopausal was associated with RRs of 1.12 (95% CI = 0.96-1.31) and 1.02 (95% CI = 0.95-1.08), respectively. No association was observed between the risk of ovarian cancer in general and BMI at 18-20 years [159].

Consistent with the association with obesity in several studies, in a prospective cohort study of 300,537 post-menopausal women done in the US, obesity was associated with a higher ovarian cancer mortality rate: compared to women with normal BMI (18.5-24.9kg/m^2), overweight (25.0-29.9kg/m^2), and obese ($\geq$30kg/m^2) women had RRs of 1.16 (95% CI = 1.04-1.30) and 1.26 (95% CI = 1.07-1.48), respectively. No trend was observed in women with a BMI <26.5kg/m^2. However, a clear trend toward higher risk with higher BMI was observed in women with a BMI $\geq$26.5kg/m^2 (P-trend = 0.001). Women with BMIs $\geq$35.0kg/m^2 had the highest risk (RR = 1.54; 95% CI = 1.12-2.24, compared to women with normal BMI). Among women who had never used ET, a statistically significant trend toward higher risk of mortality from ovarian cancer with higher BMI was observed (P-trend = 0.001). No trend was observed in women who had ever used ET (P for trend = 0.72) [161].

In a meta-analysis of 47 epidemiologic studies, 5cm greater height was associated with a 7% higher risk of ovarian cancer (RR = 1.07; 95% CI = 1.05-1.09; P<0.001). A trend of higher

risk with greater BMI was also observed in women who had never used PMH, but not in ever-users (RR = 1.10; 95% CI = 1.07-1.13; P <0.001, and RR = 0.95; 95% CI = 0.92-0.99; P = 0.02, respectively per 5kg/m^2 increase in BMI, P-heterogeneity <0.001). Based on their findings of higher risk of ovarian cancer by 1.4% per 1cm increase in height and by 1.9% per unit of BMI in women who had never used PMH, the authors concluded that, assuming a causal association, in developed countries that experience on average, a one-unit greater BMI and a 1cm greater height per decade, there would be, as a consequence, a 3% increase in the incidence of ovarian cancer [162].

2.5.9.3 Weight

In a population-based case-control study in the USA, weight in the recent past had a stronger positive association with the risk of epithelial ovarian cancer than weight at other times. Compared to women whose weight was in the lowest quartile, women with weights in the top decile had ORs of 1.5 (95% CI = 1.0-2.2) for weight at 18 years, 1.9 (95% CI = 1.2-2.9) at 30 years, and 2.1 (95% CI = 1.4-2.3) 5 years prior to interview. Compared to women with uniform weight gain, preferential gain in abdominal adiposity was associated with a statistically significantly higher risk of ovarian cancer (OR = 1.7; 95% CI = 1.1-2.6) [165]. In yet another study, recent weight and weight at 18 years of age were associated with ORs of 1.29 (95% CI = 0.98-1.70) and 1.38 (95% CI = 1.06-1.80), respectively, for 4^{th} quartile relative to 1^{st} quartile. Similar observations were made with BMI: the corresponding ORs were 1.24 (95% CI = 0.95-1.63) and 1.18 (95% CI = 0.91-1.53), respectively, for 4^{th} quartile relative to 1^{st} quartile. Height was associated with an OR of 1.26 (95% CI = 0.98-1.61) for 4^{th} quartile versus 1^{st} quartile [167].

2.5.9.4 Possible mechanisms

Genetic and environmental factors in childhood and adolescence that play a role in determining adult height may be responsible for the observed increase in the risk of cancer associated with greater height [158-160, 162]. Lack of adequate caloric intake in early life (childhood and adolescence) may lead to short stature and decreased production of growth factors including insulin growth factor (IGF) 1 [158, 159, 161]. IGF-1 stimulates cell proliferation and inhibits apoptosis [159] and, together with other growth factors, may increase the risk of cancer [161].

Several mechanisms have been proposed for a role of obesity in ovarian cancer. Obesity may lead to the development of ovarian cancer through increased oestrogen levels [158, 160, 163,

164]: obesity increases levels of oestrogen through aromatisation of androgens into oestrogens in peripheral (predominantly adipose) tissues [161, 163] and increased SHBG production [158]. This may explain the observed higher risk in obese women not using PMH and the lack of association in women using PMH whose oestrogen levels were high for this reason [161, 163]. Obesity may affect oestrogen levels in postmenopausal women via aromatisation in adipose tissue. However, overall oestrogen in premenopausal women is altered very little by higher levels due to obesity because their oestrogen is mainly produced by the ovaries [158, 165]. Obesity has also been associated with increased production of androgens [161, 163, 165], which promote cell proliferation [163].

Obesity in pre-menopausal women is associated with increased frequency of anovulation, which according to the incessant-ovulation hypothesis should result in a reduced risk of ovarian cancer. However, anovulation results in decreased production of progesterone, which may increase the risk of developing ovarian cancer [157].

High BMI is also associated with insulin resistance, hyperinsulinaemia and increased production of androgens, which promote cell proliferation [163]. Insulin stimulates production of IGF-1 and decreases production of its binding proteins (IGFBPs). Insulin and IGF-1 promote cell proliferation and inhibit apoptosis [158, 160, 168]. Insulin also inhibits the production of SHBG [168].

Leptin produced by adipocytes, is a hormone involved in weight regulation and folliculogenesis; it may also be involved in the association between obesity and the risk of ovarian cancer [163-165]. Obesity is associated with increased plasma concentrations of leptin which may stimulate angiogenesis and cell proliferation [163]. Leptin is also thought to stimulate increased production of gonadotropin-releasing hormones, which stimulates increased production of gonadotropins and increased production of ovarian oestrogen [164].

High BMI may also be a reflection of lack of physical activity. A study reported differences in the risk of ovarian cancer associated with obesity between active and inactive women (OR for obesity = 3.0; 95% CI = 1.3-6.9 and OR = 1.1; 95% CI = 0.4-3.4, respectively, P-interaction = 0.14) [166]. Contrary to this argument, studies of the relationship between physical activity and ovarian cancer have had mixed findings [169, 170]. In a population-based case-control study, recreational physical activity was inversely associated with the risk of ovarian cancer (OR = 0.73; 95% CI = 0.56-0.94, for highest versus lowest level of physical activity). Physical activity at any age was associated with some reduction of risk of ovarian

cancer [171]. Other studies did not demonstrate an inverse relationship between physical activity and the risk of ovarian cancer. On the contrary, there was a suggestion of higher risk with vigorous physical activity [169, 170].

Physical activity may decrease the production of PGE_2 which is an inflammatory mediator, and increase the production of $PGF_{2\alpha}$ which inhibits tumourigenesis [171]. Further, physical activity decreases insulin resistance (independent of body size), and therefore leads to decreased production of insulin [168, 171]. In addition, physical activity promotes immune activity which may lead to enhanced destruction of potential cancer cells. On the other hand, physical activity increases oxidative stress but also increases the production of anti-oxidants; extreme physical activity may overwhelm the anti-oxidant system [168]. The increase in risk of ovarian cancer associated with physical activity has also been attributed to detection bias: women involved in physical activity may detect symptoms earlier [169].

The association between obesity and the risk of ovarian cancer is plausible because conditions associated with obesity, such as polycystic ovary syndrome and infertility, are associated with a higher risk of ovarian cancer [158]. In addition, obesity has been likened to an inflammatory process as demonstrated by increased levels of serum adipokines [158]. Inflammation is thought to play a role in the pathogenesis of ovarian cancer.

It is difficult to compare results of studies assessing the relationship between anthropometric measures and the risk of ovarian cancer because of use of different measures, and differences in reporting of results [158, 160]. In addition, hospital-based case-control studies may have a higher number of controls with obesity than the general population, resulting in selection bias [158]. Furthermore, the ratio of muscle to fat may vary in women with the same BMI [162], active women may have more muscle than fat compared to inactive women, thus the higher risk in inactive women [166]. Despite the inconsistencies in findings, BMI and height may impact on individual's risk of ovarian cancer, and population incidence and mortality rates. Overall, current evidence leans towards higher risk of ovarian cancer with greater BMI and height.

2.5.10 GENETIC PREDISPOSITION

Genetic predisposition accounts for probably 8-10% of all ovarian cancers [37, 172]. A threefold increase in risk is observed in women with a family history of breast or ovarian cancer in a first-degree relative [172]. The majority (90%) of hereditary ovarian cancers are due to *BRCA1* or *BRCA2* mutations; the other 10% are mainly due to Lynch syndrome, which

is characterised by mutations in the DNA mismatch repair (MMR) genes: *MSH2*, *MSH6*, *MLH1*, *PMS1* and *PMS2* [37]. Compared to a lifetime risk of 1.4% in the general population [37, 173], the average lifetime risk (average penetrance up to the age of 70 years) of ovarian cancer in *BRCA1* and *BRCA2* mutation carriers are 40% and 18% respectively [37, 172-174]. In Lynch syndrome, penetrance varies according to the type of mutation and ranges from 3% to 14% [37]. There are other low-penetrance genetic variants that are associated with a higher risk of ovarian cancer [175].

Breast cancer (*BRCA*) *1* and *2* genes are located on the long arm of chromosome 17 [25, 33, 175] and the long arm of chromosome 13, respectively and are inherited in an autosomal dominant manner [25, 175]. They were first cloned in 1994 and 1995 [175].They are tumour suppressor genes and are involved in the repair of double-strand DNA breakages [25, 175]. Among patients with ovarian cancer, the prevalence of *BRCA1* mutations is 2-9% and that of *BRCA2* mutations is 1-6% [175]. Compared to the general population, women with *BRCA1* mutation are also at a higher risk of breast cancer (65-85%) [176] and endometrial cancer [177]. In addition, there is a threefold increase in the risk of prostate cancer in men in families with *BRCA1* mutations [33]. *BRCA2* mutations are also associated with a higher lifetime risk of breast cancer (45-85%) [176], melanoma, gastric, pancreas, and gall bladder cancers [177].

In *BRCA1/2* mutation carriers, ovarian cancers are usually of epithelial type and are diagnosed at a higher stage and grade compared with the general population [25, 175]. The main histological subtype of ovarian cancer associated with *BRCA* mutations is high-grade serous tumours (about 90% of cases) [25, 33, 175]. Mucinous, borderline tumours, germ-cell and sex-cord stromal tumours are not usually associated with *BRCA* mutations [175]. Ovarian cancer is diagnosed, on average, at an earlier age in *BRCA1* mutation carriers (mean age 54 years) [25] than in *BRCA2* mutation carriers (mean age 62 years) and non-familial ovarian cancer cases (63 years) [175]. Women with *BRCA1* mutations respond better to chemotherapy than those with sporadic ovarian cancers [25]; this may be due to higher sensitivity of these tumours to platinum-based chemotherapy [25, 175].

Lynch syndrome, formerly known as hereditary non-polyposis colorectal cancer (HNPCC), is characterised by a higher lifetime risk of colorectal cancer (40-60% in women and 80% for men versus 4-5%), endometrial cancer (40-60% versus 3%), and ovarian cancer (9-12% versus 1.4%) compared to the general population [176, 178], as well as biliary tract, brain,

stomach, small bowel, and ureter and renal pelvis [176, 178]. The first family with Lynch syndrome was described in 1913 by Dr Alfred Warthin and, in 1971, Dr. Henry Lynch described the syndrome [178]. Lynch syndrome accounts for about 1% of all ovarian cancer diagnoses [25]. Mucinous tumours are usually associated with Lynch syndrome [175].

Currently there are tests for genetic mutations in women who may have hereditary ovarian cancer; however, some familial cancer clusters may not have a detectable gene mutation [175]. Ideally, it is recommended that these tests be done after the age of 21 to avoid unnecessary anxiety because the risk of developing tumours associated with *BRCA* mutations or Lynch syndrome is low before this age and there will be no consequential change in management prior to this age [176]. A detailed family history of cancer (both maternal and paternal), informs the need for genetic testing, and the appropriate test [175]. A number of guidelines have been proposed for use in determining who needs genetic testing [175, 176]. The main characteristics of a hereditary-cancer syndrome include bilateralism, multiple relatives with cancer, across several generations, earlier age at diagnosis, and multiple cancers [175, 176]. Hereditary breast or ovarian cancer is characterised by the presence of a diagnosis of breast or ovarian cancer in 4 or more family members usually at an early age, or bilateral cancer of the breast [175]. Factors that may conceal the presence of hereditary ovarian cancer in the family include high prevalence of hysterectomy and/or oophorectomy performed at an early age, history of adoption, and having few female relatives [176] as well as not taking a good family history of cancer in paternal female relatives. Genetic testing for *BRCA1/2* mutations is recommended for women with a strong family history of breast and/or ovarian cancer [174, 179]. This is not recommended for the general population because of the low prevalence of *BRCA1/2* mutations (about 1 in 300-500) [179]. The test is usually performed on an affected family member (proband) and subsequently, if the test is positive, it is extended to other family members thought to be at risk [33, 175].

Incomplete penetrance of *BRCA1/2* mutations [175, 180] and Lynch syndrome strongly suggests the influence of other factors on the risk of ovarian cancer. Penetrance is influenced by type of mutation, genetic variability (position of genetic mutation), presence of other (sometimes) low-penetrance genetic mutations, and presence of risk and protective factors for ovarian cancer [175, 180].

Ovarian cancer associated with genetic mutations has been found to have a similar risk profile to that of non-familial ovarian cancer. A study of 36 carriers and 381 non-carriers

observed similar inverse associations in carriers and noncarriers with oral contraceptive use, tubal ligation, and parity. The inverse association with oral contraceptives was similar between carriers and non-carriers (OR = 0.54; 95% CI = 0.26-1.13, and OR = 0.55, 95% CI = 0.41-0.73, respectively, for use for ≥1 year versus <1 year). Similarly, there was no difference in risk of ovarian cancer associated with tubal ligation and parity between carriers and non-carriers. History of tubal ligation was not statistically significantly associated with ovarian cancer in carriers (OR = 0.68; 95% CI = 0.25-1.90), but was in non-carriers (OR = 0.65; 95% CI = 0.45-0.95). Compared to nulliparity, ≥1 term delivery (≥36 weeks gestation) was associated with an OR of 0.64 (95% CI = 0.30-1.39) in carriers and 0.46 (95% CI = 0.34-0.63) in non-carriers. A trend toward lower risk with higher parity and older age at first birth was observed in both carriers and non-carriers, although a statistically significant trend was observed only with higher parity among non-carriers. Risk of ovarian cancer for both carriers and non-carriers was not associated with BMI, history of breastfeeding, PMH use, or age at menarche. Compared to non-carriers, *BRCA1* mutation carriers were at a higher risk of serous tumours, but at lower risk of endometrioid tumours; however, a small number of carrier cases and controls were not tested for the presence of *BRCA1* mutations. In addition, some cases may have had false-negative results for *BRCA1* mutation and cases were not tested for the presence of *BRCA2* mutations [181].

Consistent with the findings of the above study, oral contraceptive use, tubal ligation, and parity were inversely related to risk in *BRCA1* and *BRCA2* mutation carriers with a strength of association comparable to that observed in the general population [182]. Among *BRCA1* mutation carriers, relative to one full-term pregnancy, a history of ≥2 full term pregnancies was associated with a statistically significant lower risk of ovarian cancer, with a statistically significant trend toward lower risk with higher parity (P trend = 0.02). Probably due to small numbers, no statistically significant inverse associations were observed in *BRCA2* mutation carriers. There was no relationship with age at first full-term pregnancy for either carrier group. History of breastfeeding was not statistically significantly associated with a lower risk of ovarian cancer in parous *BRCA1/2* mutation carriers (HR = 0.90; 95% CI = 0.61-1.32, for *BRCA1* carriers, and HR = 0.72; 95% CI = 0.27-1.91 for *BRCA2* carriers). A dose-response relationship was not observed. Ever-use of oral contraceptives was associated with a lower risk of ovarian cancer in *BRCA2* mutation carriers (HR = 0.55; 95% CI = 0.40-0.76) and there was a clear trend toward lower risk with longer duration of use (P-trend = 0.003). The inverse association diminished with longer time since last use for both *BRCA1* and 2 mutation

carriers. No relationship with age at first use was observed. A statistically significant lower risk was observed in *BRCA1/2* mutation carriers with history of tubal ligation (HR = 0.43; 95% CI = 0.24-0.75; P = 0.003) but there was no association with age at tubal ligation. No association was observed between the use of PMH or hysterectomy status and the risk of ovarian cancer among *BRCA1/2* mutation carriers. This study had low power especially for *BRCA2* mutation carriers. There was the possibility of survival bias because incident and prevalent cases were included and average time from diagnosis to interview was 6.7 years; therefore, the study might have included women with less aggressive disease [182].

In an attempt to explain the similarity in risk factors for non-familial ovarian cancer and ovarian cancer in mutation carriers, Hong et al. carried out research in which they compared the length of the different phases of the oestrus cycle in mice with and without *BRCA1* mutation. Mice with *BRCA1* mutations had longer pro-oestrus phase and a shorter metoestrus phase compared to those without *BRCA1* mutation (pro-oestrus phase: 1.63 vs 1.24 days for 3-4 months old and 1.33 vs 0.89 days for 7-8 months, and metoestrus: 1.3 vs 1.9 days). The pro-oestrus phase, which is similar to the proliferative phase in women, is associated with high oestrogen levels, whereas the metoestrus and dioestrus phases, which are identical to a woman's luteal phase, are characterised by high progesterone levels with progesterone being higher in dioestrus than metoestrus. The cycle length in mice is 4-6 days. The ratio of the pro-oestrus to metoestrus and dioestrus combined was also higher in mice with BRCA1 mutation than those without. This difference was greater in mice that were 7-8 months old than in those that were 3-4 months old, suggesting a greater effect of *BRCA* mutations at an older age. In addition, serum levels of oestrogen measured 48 hours after administration of pregnant mare serum gonadotropins (PMSG-analogous to FSH) were significantly higher in mice with *BRCA1* mutations in ovaries than those with normal ovaries (45 +/- 21 pg/ml vs 20 +/- 17 pg/ml; P = 0.002). These observations led to the conclusion that shared reproductive risk factors between *BRCA1* mutation carriers and non-carriers may be because *BRCA1* mutation and reproductive risk factors have similar effects on the menstrual cycle, thus making the presence of *BRCA1* mutations redundant in women with reproductive risk factors [183].

Family history has differing associations with the risk of ovarian cancer depending on the relationship to affected family member and cancer phenotype in the affected relative. In a population-based case-control study in California that assessed the relationship between family history of breast, ovarian, colorectal, or prostate cancer with the risk of ovarian cancer,

a statistically significant higher risk of ovarian cancer was observed in women with a history of breast and/or ovarian cancer in a first-degree relative. There was no statistically significant association between ovarian cancer and a family history of colorectal and prostate cancer in a first-degree relative. For ovarian and colorectal cancer, a statistically significant higher risk of ovarian cancer was observed when the cancer affected a sibling and not a parent, whereas for prostate- and breast-cancer family history the opposite observation was made. A relationship between the risk of ovarian cancer and a family history of cancer at other sites was observed only with lung cancer in a first-degree relative (OR = 1.73; 95% CI = 1.07-2.81). A statistically significant association was observed between a family history of breast or ovarian cancer and the risk of non-mucinous but not mucinous ovarian tumours. The inverse association between oral contraceptive use and parity and risk of ovarian cancer were not different between women with and without a family history of breast and/or ovarian cancer [184].

There was a relationship between age at diagnosis of ovarian cancer and the affected family member in a study in Sweden on *BRCA1/2* mutation carriers: diagnosis of ovarian cancer was made on average 4 years later (P = 0.009) in women with *BRCA1* mutations of paternal origin than among those with mutations of maternal origin. However, women with *BRCA1* mutations of paternal origin were diagnosed with breast cancer, on average, 4 years earlier (P = 0.017) than those with *BRCA1* mutations of maternal origin. Among *BRCA2* mutation carriers, no difference in age at diagnosis of breast or ovarian cancer was seen between women with mutations of paternal and maternal origin. Ever-use of oral contraceptives did not modify the association between age at diagnosis of ovarian or breast cancer and parental origin of *BRCA* mutations [185].

In conclusion, there are women who are at a higher risk of ovarian cancer than the general population. This risk is influenced by type of genetic mutation, parental origin of the mutated gene (paternal or maternal), and the presence of risk factors. Due to shared risk factors between women at high risk and those at average risk of ovarian cancer, similar preventive strategies can be applied, albeit with more emphasis on those at higher risk.

2.5.11 RISK ACCORDING TO HISTOLOGICAL SUBTYPE

Ovarian cancer histological subtypes are thought to be aetiologically heterogeneous and to have different risk-factor profiles. This is informed by their differences in clinical behaviour (grade & stage, invasiveness at diagnosis, response to treatment, and prognosis), descriptive

epidemiology, and genetic profiles [154, 186]. It is also not clear whether invasive and borderline tumours are a continuum or distinct diseases [28]. As will be discussed below, there is evidence to support variation in risk factors:

- between epithelial and non-epithelial ovarian tumours;
- among epithelial tumours;
- between borderline and invasive tumours;
- between mucinous and non-mucinous tumours;
- and across different histological subtypes (serous, mucinous, endometrioid, and clear-cell).

A case-control study reported differences in the association between both BMI and smoking and the risk of germ-cell tumours versus other ovarian tumours. Compared to other histological types and the general population, the ORs for germ-cell tumours in women with a BMI of >30 kg/m^2 were 4.23 (95% CI = 2.23-8.02) and 5.80 (95% CI = 3.01-11.2), respectively. Compared to other types of ovarian cancer, a statistically significant lower risk of germ-cell tumours was observed with a history of smoking (OR = 0.47; 95% CI = 0.28-0.80) [187].

Differences in risk-factor profiles between borderline and invasive epithelial ovarian tumours have been reported. In a population-based case-control study, parity was associated with a statistically significant lower risk of invasive (OR = 0.57; 95% CI = 0.33-0.98), but not borderline, tumours (OR = 0.73; 95% CI = 0.33-1.63). In addition, the lower risk with older age at first birth was statistically significant for invasive (P-trend = 0.03), but not borderline, tumours (P-trend = 0.16). Furthermore, the association with duration of use of oral contraceptives was statistically significant for invasive (P-trend = 0.001), but not borderline, tumours (P-trend = 0.28) [75].

In most studies, reproductive factors (use of oral contraceptives, parity, and ages at first and last delivery) had a stronger association with risk of non-mucinous tumours than with mucinous tumours [26, 188-190], although similar relationships for both histological types have also been reported [154]. In a population-based case-control study in Ontario Canada, statistically significant trends toward greater protection with longer duration of use of oral contraceptives and higher parity were observed for non-mucinous, but not mucinous, tumours (both borderline and invasive). For non-mucinous tumours, an OR of 0.76 (95% CI = 0.69-

0.85) was observed for each full-term pregnancy and an OR of 0.89 (95% CI = 0.85-0.93) per year of use of oral contraceptives; the corresponding ORs for mucinous tumours were 1.03 (95% CI = 0.88-1.21) and 0.98 (95% CI = 0.93-1.04) respectively [190]. These differences are thought to be due to the effect of ovulation as demonstrated by a study in which, after adjustment for ovulation, no significant differences in risk estimates associated with use of oral contraceptives or parity were observed between mucinous and non-mucinous tumours [189]. In addition, the association between lifetime ovulatory years and the risk of ovarian cancer is stronger for non-mucinous than mucinous tumours [26, 189, 191, 192]. The risk of endometrioid and serous tumours was 8% higher per ovulatory year, whereas the equivalent estimate for mucinous tumours was 3% (P-heterogeneity = 0.04) [191].

Tubal ligation is more strongly inversely associated with endometrioid tumours than with other histological types [59]. Use of PMH and higher BMI had stronger associations with endometrioid tumours than with other histological subtypes (P-heterogeneity = 0.009 and 0.06 respectively), whereas lower risk of endometrioid tumours was observed in past smokers (RR = 0.59; 95% CI = 0.39-0.90) [191]. The association between smoking and the risk of ovarian cancer was stronger with mucinous than non-mucinous tumours [26, 191]: a statistically significant higher risk of mucinous tumours was observed in current smokers (OR = 1.78; 95% CI = 1.01-3.15), whereas no association was found with non-mucinous tumours (P-difference = 0.005). A similar, but statistically non-significant, observation was made with longer duration of smoking in mucinous tumours [26].

Differences in risk factors have also been observed between type I and type II tumours [30, 31]. The association with higher parity was stronger with type I tumours than type II tumours (OR = 0.15; 95% CI = 0.11-0.21 versus OR = 0.44; 95% CI = 0.35-0.55 for ≥3 children compared to nulliparity). The inverse association with oral contraceptive use was slightly stronger for type II than for type I tumours (P-heterogeneity = 0.06). On the other hand, a history of endometriosis was associated with a statistically significant higher risk of type I tumours (OR = 1.92; 95% CI = 1.36-2.71), but not type II tumours. Similarly, tubal ligation and hysterectomy had an inverse relationship with the risk of type I tumours (OR = 0.40; 95% CI = 0.26-0.60, and OR = 0.71; 95% CI = 0.45-1.31, respectively), but were not associated with the risk of type II tumours. When classified into type I-III, very similar observations were made (P heterogeneity ≤0.04) [30].

In a study in which survival rates were used as a proxy for classification into type1/II, and type III tumours, a tumour was considered to be type III if death occurred within 3 years of diagnosis (rapidly fatal) and type I/II if survival was for more than 3 years (less aggressive); association with age, parity and use of oral contraceptives differed significantly between the rapidly fatal and less aggressive tumours. Older age was associated with a higher risk of ovarian cancer, but the relationship was stronger for rapidly fatal than for the less aggressive tumours (RR = 1.39; 95% CI = 1.29-1.49 and RR = 1.09; 95% CI = 1.03-1.16 for 5 years increase in age P-difference <0.001). Use of oral contraceptives was associated with a lower risk of both rapidly fatal and less aggressive tumours, but the inverse association was stronger for the rapidly fatal group (RR = 0.69; 95% CI = 0.58-0.82 and RR = 0.81; 95% CI = 0.74-0.89, for 5 years more use; P-difference = 0.002) [31].

Clear-cell and endometrioid ovarian tumours, which have been reported to be related to endometriosis, may have different causal pathways. In a nationwide population-based case-control study in Australia, the risk of both tumour types was similarly associated with factors that inhibit ovulation (parity, breastfeeding, and use of hormonal contraceptives); in contrast, clear-cell carcinomas seemed to have a stronger relationship with factors that affect oestrogen levels (BMI and smoking) than endometrioid tumours. Smoking was associated with a lower risk of clear-cell carcinomas (OR = 0.4; 95% CI = 0.2-0.7), but there was no relationship between smoking and the risk of endometrioid tumours. A BMI of $\geq$30kg/m^2 was positively associated with the risk of both endometrioid and clear-cell carcinomas, but the association was statistically significant for clear-cell carcinomas only (OR = 2.2; 95% CI = 1.2-4.1). On the other hand, tubal ligation was associated with ORs <1 for both tumours, but the association was statistically significant only for endometrioid tumours (OR = 0.4; 95% CI = 0.3-0.7) [193].

In an Australian-wide population-based case-control study, risk factors for serous ovarian cancer and fallopian-tube cancer were similar, but differed from those of peritoneal cancer. Compared to nulliparous women, women with a history of ever having had a term pregnancy had ORs of 0.65 (95% CI = 0.48-0.88) and 0.47 (95% CI = 0.22-1.11) for serous ovarian cancer and fallopian-tube cancer, whereas that history was associated with an OR of 1.78 (95% CI = 0.77-3.88) for peritoneal cancer. Similarly, higher parity was inversely associated with the risk of serous ovarian cancer and fallopian-tube cancer (P-trend = 0.02 and P = 0.04, respectively), but not with risk of peritoneal cancer. Each month of breastfeeding was associated with a 2% (OR = 0.98; 95% CI = 0.97-0.99; P <0.001) lower risk of serous ovarian

cancer and a 4% (OR = 0.96; 95% CI = 0.93-1.00; P = 0.03) lower risk of fallopian tube cancer, but was not associated with the risk of peritoneal cancer. In contrast, obesity was associated with a higher risk of peritoneal cancer (OR = 2.10; 95% CI = 1.29-3.44), but no relationship was observed between BMI and the risk of serous ovarian cancer or fallopian-tube cancers [194].

2.5.11.1 Possible mechanisms

The differences in risk factors by histologic type is thought to be due to differences in the cells of origin with subtypes having risk factors similar to those for cancers found in analogous sites [191, 192]. For instance, oestrogen exposure from use of PMH or high BMI has been found to increase the risk of endometrial cancer, whereas smoking lowers the risk of endometrial cancer. A similar picture is observed with endometrioid tumours [154, 186, 191]. In addition, mucinous tumours seem to have risk factors common with cervical cancer: risk of cervical cancer and mucinous tumours are not inversely associated with parity or the use of oral contraceptives [189, 190] and smoking, which has been shown to increase the risk of cervical cancer, is positively associated with the risk of mucinous tumours [26, 191]. It has also been proposed that mucinous tumours may have an aetiology distinct from other epithelial ovarian tumours [190, 192]. This may be due to their possible origin from embryonic cell remnants [190]. In addition, the ovary is also a common metastatic site, mainly for endometrial and gastrointestinal (especially colon) tumours. The differences in risk may therefore, be due to misclassification of the primary site [186]. Some ovarian mucinous tumours may be due to metastases from an occult primary colorectal tumour [190]. The observed similarity in risk factors between serous ovarian cancer and fallopian-tube cancer supports the notion that serous ovarian cancers originate from the distal fallopian tube. On the other hand, peritoneal cancer may have a different causal pathway from that of serous ovarian and fallopian-tube cancers [194]. Similarly endometrioid tumours and clear-cell carcinomas may have different causal pathways [193].

In summary, it has been demonstrated that risk factors for ovarian cancer differ by histologic subtype – and with patterns that are fairly consistent with plausible mechanisms; this strongly suggests aetiologic differences. Determination of specific risk factors and aetiology of the histological subtypes of ovarian cancer are limited by the rarity of ovarian cancer.

2.6 INCIDENCE AND MORTALITY RATES OF OVARIAN CANCER

Ovarian cancer is the 8th most common cancer in women, accounting for 3.7% of all female cancer cases and 4.2% of all deaths due to cancer in women [2]. Worldwide, more than

225,000 new ovarian cancer cases are diagnosed each year, and over 140,000 women die from this malignancy each year [1, 2]. The age-standardised incidence of ovarian cancer varies from one country to another. The highest incidence rates are observed in Scandinavian countries, the UK and North America [195, 196], whereas the lowest incidence rates are observed in China and Northern Africa [196]. Other low-incidence areas include Japan, India, Singapore, and Southern and Eastern Europe [195]. In New Zealand, in 2012 ovarian cancer was the eighth most common cancer in women, accounting for 3% of cancer registrations, and the fifth most common cause of cancer death in women, accounting for 4% of female deaths from cancer [3]. The age-standardised ovarian cancer mortality rate in NZ is ranked between those of North America and the UK [197].

Changes in incidence and mortality rates of ovarian cancer may be explained by changes in risk exposure patterns. In Western Australia, in the period 1982-1998, the age-standardised incidence and mortality rates for ovarian cancer remained unchanged. In contrast, there was an increase in the age-specific incidence and mortality rates with increase in age. The ovarian cancer incidence and mortality trends were explained by changes in parity and use of oral contraceptives (OCs): a peak in the incidence of ovarian cancer was observed in the 1924 mid-year birth cohort followed by a decline in the following birth cohorts. There was also an increase in mortality rates in successive birth cohorts with a peak in 1924, then a decline up to the mid-year birth cohort of 1949, followed by a rise in subsequent generations. A reverse pattern was observed in the age-adjusted birth rates that mirrored the changes in the incidence and mortality rate ratios. A decline in birth rates was observed in each successive mid-year birth cohorts in the period 1864-1909, followed by a rise in birth rates. A second decline in birth rates was observed in the mid-year birth cohorts of 1929-1974. In addition, OC use increased with each successive birth cohort (oral contraceptives were introduced in Australia in 1961) [196].

In a study in the UK (Wales and England) that assessed incidence (1962-1987) and mortality trends (1962-1991) in ovarian cancer in women born between 1875-1964, findings comparable to those of the study done in Australia were reported. Overall, an increase in age-specific incidence rate of ovarian cancer was observed (0.76 per annum; $P < 0.001$). The incidence rates varied by age: women aged ≥45 years had an increase of 1.32% per annum ($P < 0.001$), whereas the incidence rate for those aged <45 years decreased by 0.29% per annum ($P = 0.02$). The overall age-standardised ovarian cancer mortality rate was unchanged. A progressive increase in risk was observed in women born after the period 1875-1879 with a

peak in the 1925-1929 birth cohort. This was followed by a decline, with the lowest rates seen in the 1940-44 birth cohort, an elevated risk in the 1950-54 birth cohort, and a subsequent decline. Changes in parity showed a mirror-image pattern to the trend in risk of ovarian cancer: parity decreased from 3.3 per woman born in 1875 to 1.9 per woman born in 1910, accompanied by an increase in the risk of ovarian cancer; from 1920 to the mid-1930s, an increase in parity (from 2.0 for women born in 1920 to 2.4 for those born in the mid-1930s) paralleled a decrease in risk of ovarian cancer. In addition, since the introduction of oral contraceptives in the UK in 1960, there has been a progressive increase in the proportion of ever-users in successive birth cohorts: for women born in 1930s the proportion of ever-users was 30%, 70% for those born in 1940s, and 80-90% for those born in 1950s/1960s [198].

In a study of incidence and mortality trends in 28 European countries, in the period 1953-2000, decreases in age-specific incidence and mortality rates were observed in most Northern European countries, mainly in younger women, whereas there were increases in incidence and mortality rates in some Southern and Eastern European countries. This may be explained by a decrease in parity, largely seen in Southern and Eastern Europe, and an increase in prevalence of OC use, mainly in Northern Europe [195].

From the findings of the above studies, uptake of oral contraceptives seems to have had a greater effect on ovarian cancer trends than changes in parity. Indeed, worldwide, over 50 years, use of oral contraceptives is estimated to have prevented over 200,000 incident cases and more than 100,000 deaths from ovarian cancer [199].

Changes in diagnostic pattern may also affect the prevalence of ovarian cancer. In Sweden, in the period 1960-2005, an increase in the age-standardised incidence of borderline ovarian tumours was observed (1.0 per 100,000 woman years in 1960-1964 to 5.3 per 100,000 woman years in 2000-2005). An increase in the incidence of ovarian cancer was seen in the period 1960-64 to 1980-89, from 16.4 to 19.7 per 100,000 women-years, followed by a decline to 16.6 in 2000-05. This resulted in an increase in the proportion represented by borderline tumours in the period 1960-2005 (from 8.3% to 23.6% of all primary ovarian tumours; overall proportion for the study period was 15%). The observed increase in borderline tumours and decrease in invasive tumours may be explained by improvement in diagnostic methods and the use of OCs which confer protection against invasive but not borderline tumours [200]. In addition, a difference in prevalence of oophorectomy and

hysterectomy rates across different countries and changes in lifestyle may also influence incidence and mortality trends [195].

2.6.1 ETHNIC DIFFERENCES IN INCIDENCE AND MORTALITY RATES OF OVARIAN CANCER

In New Zealand, Polynesian women have been shown to have a higher age-standardised incidence rate of ovarian cancer than non-Pacific non-Māori women. In the period 1993-2004, the age-standardised incidence rate for Pacific women was 11.2 (95% CI = 9.5-14.0), that of Māori was 10.1 (95% CI = 8.8-11.5), and that for the non-Māori non-Pacific women was 9.4 (95% CI = 8.9-9.7) per 100,000 women. Age-standardised mortality rates followed a similar pattern with the highest rate seen in Pacific women, followed by Māori, and then non-Māori, non-Pacific women (6.3; 95% CI = 4.6-8.0, 5.8; 95% CI = 4.7-6.9, and 4.8; 95% CI = 4.5-5.0 deaths per 100,000 women respectively). Māori and Pacific women were diagnosed at a significantly younger age than non-Māori non-Pacific women (mean ages 49.8 years and 52.1 years respectively, versus 61.7 years; $P < 0.001$ for both). Māori women were diagnosed at an earlier stage of disease and at a lower grade than non-Pacific non-Māori women, although the evidence for this was weak. This study was at risk of misclassifying ethnicity because, when this was not specified, women were categorised as non-Māori non-Pacific which, may have biased the results towards the null. Māori women may have sought care for other co-morbidities, leading to early diagnosis and detection of ovarian cancer. In addition, this difference may be due to obesity which is 2.5 times more common in Pacific women than the general population. In contrast, parity which is protective against ovarian cancer, was higher in Pacific and Māori women; however, prevalence of use of oral contraceptives in the different ethnic groups, which may also affect the risk of ovarian cancer, was unknown [197].

Consistent with those findings of Firestone et al., above [197], another study done in New Zealand within the same period (1994-2002) found that the age-standardised 5-year survival rates of many cancers (including breast, cervical, colorectal, lung, prostate, and uterine) were highest in non-Māori non-Pacific women and lowest in Māori women. This difference was attributed to stage at diagnosis, and access to, cultural acceptability of, and standard of health services. In this study, use of prioritised ethnicity may have led to misclassification of ethnicity, which, again, might have biased the results towards the null. Secondly, movement of Pacific women back to the Pacific after a diagnosis of ovarian cancer may have overestimated their survival. In addition, presence of co-existing illnesses may have affected survival in Māori women [201].

Similar ethnic differences in incidence and mortality rates of ovarian cancer have been observed in other countries [196, 202]. In Australia, Indigenous women have been shown to have a 2-fold higher risk of dying from ovarian cancer compared to non-Indigenous women. This has been attributed to late stage at diagnosis and culturally insensitive health services. In addition, poorer survival in Indigenous women may be due to lack of access to early detection services, rural residence, and variation in migration patterns after diagnosis of terminal illness between non-Indigenous and Indigenous women: Indigenous women living in urban areas may move back to their homelands, which may be located in remote areas, whereas non-Indigenous women living in remote areas may move to urban areas [196].

Racial and ethnic disparities in the incidence and mortality rate of cancer have also been observed in the USA. For every stage of disease, for almost all cancer sites, African Americans have a lower 5-year relative survival rate than white Americans. Although African-American women have a 16% lower overall cancer incidence rate than white women, their mortality rate is 6% higher. This has been attributed to differences in exposure to risk factors and stage at diagnosis (African-Americans are diagnosed at a more advanced stage than white Americans), presence of coexisting illnesses, and disparities in access to and quality of health services. Variations in the age-standardised incidence rates of screen-detectable cancers (prostatic, breast, and colorectal) across different states in the USA are partly attributed to differences in screening practices [202].

In light of the above, ethnic disparities in cancer incidence and mortality are largely influenced by modifiable factors. It has been shown that with passage of time the incidence of cancer in descendants of migrants becomes similar to the local incidence rates. This points towards an environmental rather than a genetic causation [1].

2.7 SCREENING AND PREVENTION

An effective screening method has to be able to detect disease at an earlier stage at diagnosis such that treatment would result in better outcome [37, 203, 204], and reduce disease-specific morbidity and mortality [37, 204]. It also has to have high levels of sensitivity and specificity with acceptable levels of negative and positive predictive values (NPV and PPV) [203]. NPV and PPV are affected by disease prevalence and the specificity and sensitivity of the screening test. Ovarian cancer is a rare disease (prevalence of about 1 in 2,500 in postmenopausal women), which means that an ovarian cancer screening test has to have very high specificity and sensitivity in order to achieve acceptable levels of NPV and PPV. A high

false-positive rate in the screening of ovarian cancer is costly as it may lead to unnecessary anxiety and to surgical intervention with its associated morbidity [203]. A high false-negative value, of course, completely negates the value of a screening programme for each individual with a missed diagnosis.

Ovarian cancer could potentially benefit from screening because diagnosis at an early stage (stage 1) is associated with a high 5-year survival rate (95%). Development of effective screening strategies for ovarian cancer has been hampered by the low prevalence of ovarian cancer, and lack of evidence of efficacy therefore, screening for ovarian cancer does not meet criteria for population screening, but possibly may benefit those women who are known to be at high-risk of ovarian cancer [39, 40, 204].

Lack of understanding of the pathogenesis of ovarian cancer has also hampered the development of an effective preventive strategy. Understanding the natural history of disease helps in the identification of a point at which intervention is most effective in preventing disease occurrence [203].

Ovarian cancer is associated with a high mortality rate and most (>70%) are diagnosed at an advanced stage [172]. There is no screening test currently recommended for the general population [25]. Screening methods employed for women with *BRCA1/2* mutations include bimanual pelvic examination, transvaginal ultrasound, and monitoring of cancer antigen (CA)-125 levels) [25, 37]. Screening with TVS and CA-125 are inefficient (high false-positive rate) and ineffective (detects disease at an advanced stage) [174]. Despite these limitations, bi-annual TVS and CA-125 levels measurement have been recommended from the age of 35 years until oophorectomy is done [174]. The most effective preventive strategy is prophylactic bilateral salpingo-oophorectomy (PBSO) [174].

2.7.1 CLINICAL DETECTION

Use of symptoms has also been proposed for the early detection of ovarian cancer [25, 36, 205]. Urinary frequency, urinary urgency, abdominal bloating, early satiety, difficulty eating, and pelvic or abdominal pain have been proposed as symptoms that suggest the presence of ovarian cancer, which should therefore be considered as a possible diagnosis in subsequent investigations [25, 36, 205]. A positive symptom index, defined as the occurrence of any one of the above 6 symptoms >12 times in a month for less than a year, was found to have a sensitivity of 56.7% for early stage, 79.5% for advanced disease, and 80% for unstaged disease. The sensitivity and specificity were both 86.7% for women who were <50 years,

whereas for women who were ≥50 years the sensitivity and specificity were 66.7% and 90% respectively [205]. There is the possibility that such use of symptom indices aids in earlier detection of advanced disease and not detection of disease at an early stage. Early detection of advanced disease may be beneficial because there is better success at optimal surgical debulking and early intervention may also improve quality of life of the patients [206].

In a population-based case-control study in California, only 16% of women diagnosed with ovarian cancer were asymptomatic at the time of diagnosis. In addition, asymptomatic women were at an early stage compared to the symptomatic women (65% versus 51%; P = 0.01). Relative to other tumour types (mucinous-10%; endometrioid-19%; and clear cell-14%), the majority (55%) of asymptomatic women diagnosed with serous tumours had advanced disease (P = 0.01). The short duration of symptoms and advanced disease at diagnosis of serous tumours may point to rapid disease progression as opposed to a delay in diagnosis. Diagnosis by symptoms may therefore fail to detect a high proportion of early serous tumours. In addition, symptoms were found to differ by histological subtype of EOC. Abdominal distension was a common presentation in women diagnosed with mucinous tumours (60%), followed by serous tumours (43%), and endometrioid tumours (26%). History of abnormal vaginal bleeding was more common in women with endometrioid tumours (19%) than in those with serous tumours (7%), whereas bowel symptoms were more commonly reported by women with serous tumours (47%) compared to non-serous (19-32%). There were also differences in symptom presentation according to tumour grade, stage, and age, but not with ethnicity. However, it is important to note that stage and grade were influenced by the histological type of tumour. The majority of serous tumours (69%) were diagnosed at an advanced stage compared to 13-38% of non-serous tumours. The majority of serous (75%) and clear cell (72%) tumours were high-grade compared to endometrioid (38%) and mucinous (13%).There was also a positive relationship between tumour grade and stage at diagnosis, with high-grade tumours being diagnosed at an advanced stage (61%) relative to low-grade tumours (27%), although even here, influence of histological subtype was observed, with most of the non-serous high-grade tumours still being diagnosed at an early stage [38].

Pelvic examination can be used to detect adnexal masses; however, it has low sensitivity [23, 25]. Pelvic examination detects about 1 in 10,000 ovarian cancers in asymptomatic women [25], and fails to detect 10% of adnexal masses of 10 cm in size [23].

2.7.2 ULTRASOUND

Ultrasound has limited value in detecting early-stage ovarian cancer [25]. However, in a study in which surveillance of asymptomatic women using annual transvaginal ultrasound was done, the 5-year survival rate of screened women after a diagnosis of invasive EOC was significantly better than that of unscreened women (76% +/- 6.6% Versus 53.7% +/-2.3%; P <0.001). Use of TVS as a screening test had a sensitivity of 86.4% and a specificity of 98.8%. Ovarian cancer was also detected at an early stage (47% in stage I and 23% in stage II); the improved survival observed in this study was due to detection at an early stage (which may have been partly due to lead-time bias). Follow-up began at 25 years of age for women with a strong family history of breast or ovarian cancer (genetic testing was not routinely done in this study) and at 50 years of age for women at average risk. The mean follow-up period was 5.8 years. Patients underwent standard treatment with surgery followed by 6 cycles of platinum-based chemotherapy (carboplatin plus taxanes) [204].

Use of TVS as a screening test is limited by its low PPV [37, 204], which is more marked in premenopausal women due to frequent formation of functional cysts [37]. In the above study, the negative predictive value of a normal ultrasound was 99.97%, and the positive predictive value was 14.5% (6.9 surgical operations per ovarian cancer detected) [204].

2.7.3 BIOMARKERS

CA-125, a large transmembrane glycoprotein, was first identified in human ovarian cancer cell lines in 1981 by Bast and colleagues [203, 207]. In 2001, the gene encoding CA-125 (*MUC16*) was cloned [203, 207]. Currently, CA-125 is used in detection as well as monitoring of treatment and follow-up among patients with ovarian cancer [207, 208]. The biologic function of CA-125 is unclear [203, 207]; however, it is thought to promote ovarian cancer tumourigenesis [207]. The normal cut-point for CA-125 plasma level is 35 IU/ml [207, 208].

A cut-point of 35 IU/ml is associated with a high false-positive rate in pre-menopausal women, which may be of concern particularly in high-risk women [208]. CA-125 is produced by many tissues including epithelia of female reproductive tract, pleura, pericardium, peritoneum, lung, pancreas, breast, stomach, and gall bladder. In addition, CA-125 is elevated in certain benign conditions such as endometriosis, fibroids, pregnancy, PID, pericarditis, pleurisy, pancreatitis, liver disease, TB, and peritonitis, and also in malignancies of other sites such as breast and gastrointestinal cancers [207]. It is elevated in women with EOC [23, 25], but varies according to histological subtype (highest in serous and lowest in mucinous) [25].

In addition, use of CA-125 as a screening test for women with Lynch syndrome may not be as effective as in *BRCA1/2* mutation carriers because tumours in this group of women are usually non-serous [172]. The sensitivity of CA-125 for the detection of stage I ovarian cancer is low (25-50%) [37].

Normal levels of CA-125 are also affected by certain clinical and demographic factors and are higher in premenopausal than postmenopausal women [207, 208]. In a prospective study in the US in which levels of CA-125 were measured in high-risk women (women with a strong family history of breast or ovarian cancer), premenopausal women had a significantly higher 98^{th} percentile cut-point of CA-125 than postmenopausal women (52 IU/ml versus 36 IU/ml; $P<0.001$). In pre-menopausal women, use of oral contraceptives was associated with a significant reduction in the cut-point (98^{th} percentile) of CA-125 (reduced to 39 IU/ml; $P <0.001$) [208].

Other factors that were associated with lower CA-125 levels in pre-menopausal women included smoking, irregular periods, *BRCA1/2* mutation, Ashkenazi Jewish heritage, and a family history of ovarian cancer. Factors that were associated with lower CA-125 levels in post-menopausal women included history of bilateral oophorectomy, hysterectomy, black race, and history of use of fertility treatment. Factors that were associated with levels of CA-125 (in both pre-and post-menopausal women) had an additive effect. The authors concluded that in order to have equivalent false-positive rates between pre- and post-menopausal women, a cut-point of 35 IU/ml should be used for post-menopausal women, 50 IU/ml for pre-menopausal women, and 40 IU/ml for premenopausal women on oral contraceptives [208]. However, this may decrease the sensitivity of CA-125 as a screening test and therefore result in failure to detect ovarian cancer at an early stage [207]. Individualisation of CA-125 levels is further complicated by the effect of more than one factor [207].

The Risk of Ovarian Cancer Algorithm (ROCA) takes into account the variations in CA-125 levels. Women with CA-125 levels within the normal range but rising are considered at high risk, whereas those with CA-125 levels above the normal range but static are deemed to be at low risk. In estimating an individual's risk of ovarian cancer, ROCA utilizes an algorithm that includes a woman's age-specific risk of ovarian cancer and CA-125 dynamic profile. Using ROCA the sensitivity of CA-125 is 86% and the specificity is 98% [207].

The risk of malignancy index (RMI) first proposed by Jacob et al. [209] has also been used for the pre-operative diagnosis of ovarian cancer. The scoring is derived from the product of

the absolute level of plasma CA-125 levels, menopausal status (a score of 1 for pre-menopause and 3 for post-menopause), and ultrasound features (scores of 0, 1, and 3) [209, 210]. Using a cut-point of 200 to indicate malignancy a sensitivity of 90% and a specificity of 89% in discriminating between benign and malignant disease has been reported. A high positive predictive value (96%) and negative predictive value (78%) were also observed. Furthermore, RMI was more accurate in detecting cancer at an early stage than any of its individual components. RMI can be used in less-specialised units and helps avoid unnecessary surgery [210].

The findings of the UK Collaborative Trial of Ovarian Cancer Screening (UKTOCS), a randomised controlled trial involving 202,638 post-menopausal women (50-74 years of age), suggest that use of CA-125 in combination with pelvic ultrasound may reduce mortality from ovarian cancer in this group of women. In that study, participants were randomised into 3 groups: no screening, ultrasound screening (USS), and CA-125 with ultrasound as a secondary test (multimodal screening [MMS]). Over years 0-14, a reduction in mortality from ovarian cancer of 15% (95% CI -3 to 30) was observed in the MMS group and 11% (-7 to 27) in the USS group, which were not statistically significant. However, for years 7-14, there was a reduction in mortality of 23% (95% CI = 1-46) in the MMS group and 21% (-2 to 42) in the USS group. MMS had a sensitivity of 84% (95% CI = 79-88) and USS 73% (66-79). There was a false-positive rate of 1% in the MMS group and 3.2% in the USS group; 14 and 50 unnecessary surgeries per 10,000 screens respectively. There was no difference in the incidence of ovarian cancer in the three groups [211].

Biomarkers other than CA-125 have also been evaluated for use as screening tests for ovarian cancer [37, 203]. Biomarkers have been shown to differ according to epithelial ovarian cancer histological subtype, which may be one of the reasons why it is difficult to establish a single biomarker for ovarian-cancer detection [43]. Despite the presence of other biomarkers, CA-125 is still the best available and has been described as “the best of a bad lot” [203].

2.7.4 CHEMOPREVENTION

Oral contraceptives (OCs) have been shown to be associated with about a 50% reduction in the risk of ovarian cancer [37, 174] and the inverse association lasts up to 20 years after cessation of use [172]. However, there are concerns about increased risk of breast cancer with use of oral contraceptives as a chemopreventive agent in premenopausal women [37, 172, 174]. This has led to the suggestion that women at high-risk of ovarian cancer should use oral

contraceptives for 3-5 years when they are <25 years of age (at this age, the incidence of breast cancer is low) [37]. Use of oral contraceptives solely for the prevention of ovarian cancer has the disadvantage of also preventing pregnancy [174].

2.7.5 SURGICAL PREVENTION

The most effective preventive strategy for women with *BRCA1/2* mutations is prophylactic bilateral salpingo-oophorectomy (PBSO), also known as risk-reducing salpingo-oophorectomy (RRSO) [37, 174, 208]. Ovarian cancer is diagnosed, on average, at an earlier age in *BRCA1/2*-mutation carriers than in the general population [172]. A diagnosis of ovarian cancer is made in about 3% of *BRCA1*-mutation carriers aged ≤40 years and this rises to 21% at ≤50 years [173]. The recommended age for PBSO is 35-40 years in *BRCA1*-mutation carriers and 40-45 years in *BRCA2*-mutation carriers. Oophorectomy done in premenopausal women has the added advantage of decreasing the incidence of breast cancer [37].

Prophylactic bilateral salpingo-oophorectomy has been shown to decrease the risk of tubal/ovarian cancer by 85-95% and that of breast cancer by ≥50%. It also lowers disease-specific mortality from ovarian or breast cancer [174]. Occult ovarian, peritoneal, or tubal cancers have been reported in 2-18% of cases at the time of surgery [37, 173, 174]. Therefore, care is recommended and needed at the time of surgery in order to minimise the risk of spread. In addition, peritoneal washing for cytological examination and careful sectioning of ovaries and tubes during histological examination is recommended [37, 173]. In *BRCA1/2*-mutation carriers BSO may be sufficient; however, in women with Lynch syndrome, hysterectomy is required due to the elevated risk of endometrial cancer [37].

The risk of peritoneal cancers is not totally eliminated by PBSO [172-174]. A residual risk of 1-4% is present [173, 174] and is more common in *BRCA1*- than in *BRCA2*-mutation carriers [174]. This is thought to arise as a result of malignant transformation of peritoneal tissue or from occult cancer that is not detected at the time of surgery and therefore left untreated [173]. Despite this, surveillance with CA-125 after surgery is not recommended [173]. Overall, the incidence of ovarian, tubal, or peritoneal cancer is decreased from 1% to 0.2% per year [173].

Prophylactic total hysterectomy and bilateral salpingo-oophorectomy (THBSO) after the age of 40 is a preventive option for women with Lynch syndrome. The timing is recommended on the basis of the risk of ovarian and endometrial cancers up to this age: ≤2% for endometrial

cancer and ≤1% for ovarian cancer. Due to the possibility of occult endometrial cancer, endometrial biopsy is recommended prior to surgery [175, 212]. Women with Lynch syndrome undergoing colon surgery benefit from THBSO done at the same time. There is the possibility of peritoneal cancer after risk-reducing THBSO [212]. In addition, THBSO does not eliminate the risk of other malignancies associated with Lynch syndrome [175]; therefore, screening for colorectal cancer should continue [37].

The downside to oophorectomy prior to menopause is that it leads to surgical menopause, which results in hormonal deprivation with its attendant complications [213]. To mitigate the complications associated with premature menopause induced by RRSO, women may use PMH for 2-3 years [174, 175]. Use of PMH in women with no prior history of breast cancer does not negate the reduction of risk of breast cancer accorded by oophorectomy [174, 175, 213]. Use of PMH is contraindicated in women with prior history of breast cancer [173]. PMH should only be used up to the age of 50 years (approximate age at menopause); beyond this it has been shown to reduce life expectancy even in women with history of mastectomy [175].

In a prospective study in Toronto, women with *BRCA1/2* mutations who underwent PBSO completed questionnaires before and after surgery; surgery prior to menopause was shown to affect quality of life. Increase in vasomotor symptoms and a fall in sexual function were observed. This was substantially, but not fully, alleviated by use of PMH. However, satisfaction with the decision to undergo surgery was high (mean 4.55 out of 5), with no difference in the level of satisfaction between pre-and post-menopausal women (4.53 vs 4.61, P = 0.63) [213]. Women with surgical menopause are also at higher risk of cardiovascular disease, and loss of bone density; the latter, may be improved by PMH [173].

Bilateral salpingectomy without oophorectomy has been suggested for women who desire to conserve their ovaries [173, 214]. However, the effect of this on decreasing the risk of ovarian cancer risk is unknown. In addition, it does not decrease the risk of breast cancer [173].

The appropriate measure of an effective screening method is its ability to decrease disease-specific mortality [37, 203]. CA-125 measurements and annual TVS have failed to detect ovarian cancer at an early stage and failed to decrease mortality attributed to ovarian cancer [37, 207]. Screening of the general population for EOC is currently not recommended [172].

Our current lack of effective screening results in the diagnosis of ovarian cancer at an advanced stage, at which time the cost of treatment is high and the survival rate is low [204].

2.8 PROGNOSIS

Ovarian cancer is the most fatal gynaecological cancer, with a 5-year survival rate of <50% [63, 137, 163]. This is partly due to lack of understanding of the pathobiology of ovarian cancer and to its advanced stage at diagnosis [127]. Stage [215-218], histological subtype [215], and grade [216, 217, 219] are important prognostic determinants of ovarian cancer. In a population-based prospective cohort study in Australia of women aged 18-79 years with incident invasive EOC, the most important prognostic indicator was stage at diagnosis. Compared with stage I disease, higher stage was associated with progressively lower survival with an HR of 10.1 (95% CI = 5.9-17.2) in women with stage IV disease [216]. Similarly, in a prospective cohort study of women with stage I or II ovarian cancer, a combination of sub-stage and histological type of tumour were found to be important predictors of outcome. Having stage IA or IB mucinous or endometrioid tumours was associated with a disease-specific survival at 10 years (DSS10y) of 95%, compared to an overall DSS10y of 70.1%. Among women with clear-cell carcinoma, those with stage IA and IB had a DSS10y of 87%, and those with stage IC-II, 66%. The prognosis was poor for those diagnosed with high-grade serous tumours (DSS10y = 57%). Based on their observations, the authors proposed that adjuvant chemotherapy and extensive surgical staging may not be required for stage IA and IB mucinous and endometrioid tumours. In addition, unilateral oophorectomy may be done for those with stage IA mucinous or endometrioid tumours [215].

Stage and grade have also been shown to influence survival of non-epithelial ovarian cancer. In a study of women diagnosed with germ-cell tumours in a hospital in China, factors that had a significant influence on survival were stage of disease (P = 0.003), nuclear atypia (P = 0.036) and increased mitotic rate (P = 0.002). Slight nuclear atypia was associated with a 10-year survival proportion of 76% compared to 42% for marked atypia [219]. Use of grade in determining prognosis is limited by the low reproducibility of grade; histological typing has better reproducibility than grade. In addition, grade may be a proxy for type. However, grade does not accurately represent the underlying biological differences of the various histological types [215].

In line with their positive association with the occurrence of ovarian cancer, obesity [217, 220] and smoking [216, 221] have been shown to have a negative influence on survival. In a

study by Previs et al. of women with LGSC, having a BMI of ≥30 kg/m^2 was associated with a statistically non-significant poorer overall survival (OS) (HR = 2.08; 95% CI = 0.93-4.66; P = 0.07): a 1.4-fold increase in the risk of death from LGSC with every 5 kg/m^2 increase in BMI, and 2.1-fold increase in the risk of death with every 10 kg/m^2 increase in BMI were reported. However, obesity did not influence DSS or PFS (8.4 vs 12.2 years; P = 0.5, and 1.5 vs 2.6; P = 0.2, respectively, for obese versus non-obese women) [220]. In a prospective cohort study in Sweden, being overweight at 18 years and at 1 year before diagnosis were both associated with higher risk of death from stage I and II disease (HR = 2.2; 95% CI = 1.0-4.7, and HR = 1.9; 95% CI = 1.1-3.4, respectively), but were not associated with survival for stage III or IV disease. In addition, obesity throughout adulthood was associated with poor survival in women with early-stage (stage I or II) disease (HR = 1.7; 95% CI = 1.1-2.6). History of engaging in physical activity for at least 2 hours per week at 18-30 years of age afforded a survival advantage from stage I or II epithelial ovarian cancer (HR = 0.5; 95% CI = 0.2-1.0), but was associated with higher risk of death from late stage (III and IV) disease (HR = 1.4; 95% CI = 1.0-2.0) [217].

A prospective study of patients with invasive epithelial ovarian cancer reported a higher risk of death from ovarian cancer in current smokers than in never-smokers (HR = 1.36; 95% CI = 1.01-1.84) and this was more evident in women with advanced disease (HR = 1.58; 95% CI = 1.15-2.16). Higher pack-years of smoking were associated with a higher risk of death (HR = 1.94; 95% CI = 1.41-2.66, for >30 pack years versus never smokers) and this was also more apparent in women with advanced tumours [221].

Reproductive factors including parity, breastfeeding, use of oral contraceptives, use of PMH, age at first and last delivery, and age at menarche are important predictors of ovarian cancer incidence; however, studies assessing their association with the risk of mortality from ovarian cancer have had null findings [217, 218]. Nonetheless, in one study reproductive factors had no influence on survival with the exception of breastfeeding: among parous women, history of breastfeeding was associated with better survival (HR = 0.74; 95% CI = 0.55-0.98). There was no association with duration of breastfeeding (P trend = 0.72) or duration since cessation of breastfeeding. In addition, breastfeeding was not associated with lower stage at diagnosis or lower tumour grade [216].

On the other hand, tubal ligation has been associated with poor outcome following ovarian cancer diagnosis [218]. In a prospective cohort study in China of 202 women aged < 75years

with histologically confirmed epithelial ovarian cancer, history of tubal ligation was associated with a statistically significantly higher risk of mortality from ovarian cancer (HR = 1.62; 95% CI = 1.01-2.59; P = 0.04). However, there was no association with hysterectomy [218]. A null association between tubal ligation or hysterectomy and mortality from ovarian cancer has also been reported [216].

Other prognostic indicators are:

- age (there is a better prognosis with diagnosis at younger ages) [216];
- chemotherapy [25, 219] (chemotherapy improves survival of women with stage III disease (with or without optimal cytoreduction) and, to some extent, stage IV disease [25]); and
- residual tumour size following cytoreductive surgery (optimal cytoreduction is associated with better survival) [216, 220].

To explain the observed association between smoking and the risk of death from ovarian cancer, it has been proposed that carcinogens found in tobacco may change tumour characteristics to favour more aggressive tumours. The carcinogens may also change hormone levels or affect the immune function of the patient, thereby creating a favourable environment for tumour progression [221].

Because of the observed effects of obesity on overall survival (OS) but not PFS and DSS, one possibility is that higher mortality is due to co-existing illnesses. Obesity increases the risk of diabetes mellitus and cardiovascular disease. In addition, obesity is associated with increased insulin resistance, increased levels of oestrogen and androgens, and chronic inflammation which may alter tumour behaviour. In addition, increased levels of leptin associated with obesity may result in enhanced angiogenesis due to induction of VEGF [220].

Sterilised women may still be at risk of serous tumours that originate from the fimbria and are usually diagnosed at an advanced stage and therefore have a worse prognosis. For instance, in the study by Zhang et al., 57% of women with history of tubal ligation had serous tumours, compared to 34% of those without tubal ligation. This may explain the higher risk of death from ovarian cancer associated with tubal ligation [218].

In summary, age, histological type of tumour, residual tumour size, stage, and grade are predictors of survival from ovarian cancer. These factors are not modifiable. Other factors that may increase risk of death are smoking and obesity; therefore, lifestyle change after

diagnosis may be beneficial. Although reproductive factors are associated with the incidence of ovarian cancer, they do not seem to influence survival.

2.9 SUMMARY

Ovarian cancer is mostly diagnosed at an advanced stage with a resulting poor outcome. The primary reasons are lack of early-detection methods and lack of an understanding of its pathobiology. In addition, ovarian cancer seems to be a heterogeneous disease that requires screening and management methods specific to its histological subtypes.

Currently, there are no screening tests recommended for women at average risk of ovarian cancer. Surveillance tests employed in high-risk women include monitoring of CA-125 serum levels, transvaginal ultrasound, and pelvic examination. None, either individually or in combination, has consistently been shown to detect ovarian cancer at an early stage or to reduce mortality from ovarian cancer. Furthermore, the high false-positive rates associated with these tests, result in anxiety and unnecessary surgical interventions. Oral contraceptives, which have been shown to decrease the risk of ovarian cancer by up to 50%, have been proposed for use as a chemopreventive agent. With the recent proposal that the most aggressive histological type, serous tumours, arise from the fimbrial end of the fallopian tubes, salpingectomy without oophorectomy has also been proposed as an interim risk-reducing strategy for women who wish to conserve ovarian function, but its effect in decreasing risk of ovarian cancer is unknown. The most effective ovarian cancer preventive strategy in women at high risk is risk-reducing salpingo-oophorectomy (RRSO) after the completion of child bearing, although this has the disadvantage of inducing early menopause.

The pathogenesis of ovarian cancer is not fully understood, and it has been suggested that ovarian tumour cells arise from extra-ovarian sites with secondary involvement of the ovary. The proposed pathogenetic pathways include the incessant-ovulation hypothesis, the gonadotropin hypothesis, inflammation, and the hormonal hypothesis (changes in the levels of oestrogens, androgens, and progesterone). In keeping with this, the proposed mechanisms of effect of most of the predictors of ovarian cancer influence one or more of these pathways. In addition, the different histotypes of epithelial ovarian tumours have different risk profiles, which may be explained by their different cells of origin.

Studies assessing the association of various factors with the risk of developing ovarian cancer have had mixed findings in most instances. In addition, there is evidence that risk factors differ by histological type. Nonetheless, when ovarian cancer is viewed as a single disease

entity, non-modifiable risk factors include genetic predisposition, height, endometriosis, infertility, age, early age at menarche, and late age at menopause, whereas, modifiable risk factors are talcum use, obesity, use of PMH, smoking, and alcohol use. Protective factors include parity, use of oral contraceptives, breastfeeding, tubal ligation, oophorectomy, and hysterectomy.

A better understanding of modifiable risk factors of ovarian cancer will help in developing preventive strategies and in understanding its pathogenesis. Contraceptives, a potentially modifiable factor, are widely used by women of reproductive age. With the observation that use of oral contraceptives confers reasonable long-term lower risk, it is conceivable that other contraceptives with similar hormonal content and mechanisms of contraception confer protection from developing ovarian cancer. In addition, other contraceptives that affect the proposed pathogenetic pathways of ovarian cancer may also influence risk. In Chapter 3, available contraceptives, their mode of action, and local and systemic effects will be discussed. In Chapter 4, their association with the occurrence of ovarian cancer will be discussed.

CHAPTER 3: CONTRACEPTION

In Chapter 2, factors associated with ovarian cancer and their hypothesised mechanisms of effect were discussed. Their associations with the risk of ovarian cancer were mainly attributed to hormonal changes, ovulation inhibition, and local effects on the female reproductive tract and ovary. In this chapter, an overview of available contraceptives, and their modes of action are presented. The influence of contraceptive use on the occurrence of ovarian cancer is expected to be through their local and systemic effects.

3.1 INTRODUCTION

The term contraception includes all measures, temporary or permanent, designed to prevent pregnancy [222]. Available contraceptives include hormonal contraceptives, intrauterine contraceptive devices, sterilisation, barrier methods, and natural contraception. There have been substantial advances in the development of contraceptives, including transitions from high-dose to low-dose combined oral contraceptives, and from inert to copper-bearing and levonorgestrel-releasing intrauterine contraceptive devices. The idea is to attain maximum efficacy while decreasing adverse effects. The use of contraceptive methods, with the exception of male and female sterilisation, usually does not result in an irreversible change in fertility [17].

3.2 NORMAL FEMALE AND MALE REPRODUCTIVE FUNCTION

The female reproductive function occurs in a cyclic manner, termed the "menstrual cycle", regulated by the hypothalamo-pituitary-ovarian axis. The hypothalamus produces gonadotrophin releasing hormone (GnRH) which stimulates the production of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary gland. LH and FSH act on the ovary to induce follicular maturation and production of hormones. These hormones include steroids (oestrogens, progestogens and androgens), peptides (activin, follistatin, and inhibin) and growth factors. These hormones usually exert a negative feedback effect on the pituitary and the hypothalamus to decrease the production of gonadotropins (LH and FSH) and GnRH. However, around the time of ovulation, oestrogen triggers a positive feedback loop with the pituitary gland causing an increase in LH production. The LH surge is necessary for ovulation to occur. After ovulation, the corpus luteum is formed, which continues secreting oestrogen, progesterone, and inhibin. If pregnancy does not occur, the corpus luteum degenerates after about 2 weeks, the oestrogen and progesterone levels decline, menstruation ensues, and a new cycle begins [20, 21, 45].

As the development of follicles, ovulation, and formation of the corpus luteum occur, changes in the endometrium also take place. Oestrogen induces proliferation of the endometrium in the first part of the cycle (proliferative phase/follicular phase). Progesterone, mainly produced by the corpus luteum, causes changes in the endometrium in the second part of the cycle (secretory phase/ luteal phase). These changes prepare the endometrium for implantation in case fertilisation takes place [20, 21, 45].

The luteal phase of the menstrual cycle is usually fairly constant, lasting about 14 days. Therefore, the duration of the follicular phase is responsible for the differences in cycle length [20, 21, 45]. The first day of menstruation is the beginning of the menstrual cycle [45]. After ovulation, the ovum remains viable for about 24 hours; therefore, for pregnancy to occur, intercourse has to take place around the time of ovulation, usually five days before ovulation through the day of ovulation [20, 45].

Other than the effects on the ovary and endometrium, oestrogens and progestins (synthetic hormones that have effects similar to those of progesterone) exert effects on other organs including the fallopian tubes, breasts, bones, kidneys, cervix, and liver. The effects on the fallopian tubes are similar to those seen in the endometrium. Oestrogens cause deposition of fat and development of stromal tissue and ducts in the breast, whereas progesterone causes development of the lobules and alveoli. Oestrogen accelerates bone growth, induces closure of the epiphyses, and prevents bone resorption by inhibiting osteoclastic activity. In the kidneys, it promotes sodium reabsorption, therefore causing water and sodium retention in the body [20, 21]. Oestrogen increases the production of mucus by the endocervical glands, makes the mucus more watery, and increases its elasticity; these changes allow the spermatozoa access to the upper female reproductive tract. Progesterone decreases the quantity of cervical mucus and makes it viscous, making it impermeable to sperm [21]. Oestrogen also increases the production of steroid-binding globulins by the liver; stimulates increased production of angiotensinogen; increases the levels of high density lipoproteins (HDLs) and very low density lipoproteins (VLDLs), and lowers the plasma levels of low density lipoproteins (LDLs) and cholesterol [21]. In addition it increases the production of clotting factors, fibrinogen, and factors II, VII, IX, X, and XII [45]. In contrast, progesterone increases the level of cholesterol and LDL, and decreases the level of HDL. It also increases the basal body temperature [21]. Steroid hormones are metabolised in the liver and are therefore contraindicated when there is active liver disease [21, 45]. Synthetic oestrogens and progestins are used in oral contraceptives in order to reduce catabolism by the liver [20].

Spermatogenesis occurs in the seminiferous tubules and takes about 74 days. About 120 million spermatozoa are produced in the human testes each day. They are stored mainly in the vas deferens and, to a lesser extent, in the epididymis, maintaining viability for up to 30 days. During ejaculation, the spermatozoa are emptied into the epididymis which connects to the vas deferens. The seminal vesicles empty into the prostatic end of the ampulla of the vas deferens which leads to the ejaculatory duct. The ejaculatory duct also receives the contents of the prostate gland from the prostatic duct. The ejaculatory duct connects to the urethra, which is the final link between the testes and the exterior. Semen is composed mainly of fluid from the seminal vesicles (about 60%), and prostatic fluid (about 30%). Spermatozoa and fluid from the vas deferens – which contains hormones (including testosterone and oestrogen), enzymes, and special nutrients – constitute 10% of the semen. Spermatozoa are stored in an inactive state. After ejaculation they become motile and undergo maturation in a process known as capacitation. The spermatozoa can survive only for 24 to 48 hours in the female reproductive tract [20].

For pregnancy to occur, ovulation, transport of the fertilised ovum into the uterine cavity, and a favourable endometrium for implantation, are all needed. In addition, sperm of adequate number and quality must be deposited at the cervix around the time of ovulation [45]. Tubal transport is dependent on a fluid current of epithelial secretions, tubal cilia, and contractions of the fallopian tube [20]. Contraception is achieved by inhibiting ovulation, preventing fertilisation of the ovum, or by inhibition of implantation.

3.3 HORMONAL CONTRACEPTION

Hormonal contraceptives contain synthetic oestrogens and progestins, in combination or progestins alone. Their contraceptive action is through the effects of these hormones. These hormones are also responsible for the unwanted effects and the health benefits of these contraceptives.

3.3.1 COMBINED HORMONAL CONTRACEPTIVES (CHCS)

Combined hormonal contraception provides an oestrogen and a progestin; they include combined oral contraceptives, combined injectable contraceptives, vaginal ring (Nuva Ring) and transdermal patch (Evra) [17, 45].

3.3.1.1 Combined oral contraceptives

In combined oral contraceptives (COCs), the commonly used progestins are the estranes (norethindrone and norethindrone acetate), the gonanes (levonorgestrel, desogestrel, and

norgestimates), and the spironolactone analogue drospirenone [23, 222]. Progestogens used in COCs have different androgenic, oestrogenic and progestational activity [223]. The oestrogens are principally confined to either ethinyl oestradiol or its 3-methyl ether, menstranol. When first developed, the two principal regimens of COCs were combined and sequential. In the combined regimen all active pills contain both oestrogen and progesterone [23]. In the sequential regimen, oestrogen-only pills are taken during the first part of the cycle followed by pills containing both oestrogen and progesterone [222]. The sequential method has been largely abandoned because several studies showed a high incidence of endometrial cancer in women using this method of contraception [23, 222]. Currently, combined oral contraceptive pills are available in monophasic, biphasic, and triphasic preparations. Monophasic preparations have the same amount of oestrogen and progestin in each active pill; biphasic preparations contain two different dose combinations; and triphasic preparations have three different dose combinations [45, 224]. In the most commonly used combined method, pills containing both oestrogen and progestin are taken each day for 21 days, followed by 7 days of placebo pills, during which time most women experience withdrawal bleeding. Over the years, the oestrogen content has been reduced by a factor of 3- to 4-fold, such that the current dose of ethinyl oestradiol ranges between 20 and 35 μg. Similarly, the progestin content has been substantially reduced [23, 222]. This has decreased the occurrence of unwanted effects without change in efficacy [17, 223]. Reduction of oestrogen to 20μg has reduced cardiovascular mortality in OC users by 60% [223].

The probable mechanisms of contraception are:

- Inhibition of ovulation: both hormones act synergistically on the hypothalamo-pituitary-ovarian axis to inhibit ovulation. The release of GnRH from the hypothalamus is prevented through a negative feedback mechanism. Consequently, there is no rise in FSH and LH during the first half of the cycle and there are no mid-cycle alterations in FSH and LH levels; as a result, the growth of the dominant follicle and subsequent ovulation does not occur [23, 45, 222, 224].
- Inhibition of endometrial maturation: the progestogen component makes the endometrial lining less receptive to implantation by producing static endometrial hypoplasia (there is stromal oedema, decidual reaction, and regression of the glands) [23, 45, 222-224].

- Thickening of cervical mucus: the progestogen alters the character of the cervical mucus resulting in less sperm penetration [23, 45, 222-224].
- Interference of tubal function: the progestogen interferes with motility and secretions of the fallopian tubes, thereby altering tubal transport of both sperm and oocyte [23, 222-224].

Medical complications may arise from use of COCs. Hypertension is due mainly to the oestrogen component, which causes an increase in plasma angiotensinogen. Vascular complications, including thromboembolic disease, myocardial infarction (MI), and stroke that may arise as a result of using COCs, are also attributed to the oestrogen component. Use of current COCs roughly triples a user's risk of venous thromboembolism (VTE), including pulmonary embolism (PE). VTE is enhanced by risk factors such as pre-existing hypertension, diabetes, obesity, age (higher risk in those over 35 years, especially smokers), recent leg trauma, pelvic surgery, stasis (but not varicose veins), and the presence of germline mutation in the clotting system known as factor V Leiden. MI and stroke are rare conditions; they occur among COC users only in the presence of risk factors such as hypertension, diabetes, severe dyslipidemia, smoking, and age over 35. Cholelithiasis, and benign liver tumours are occasionally noted with COC use [17, 23, 45, 222, 223]. The relationship between the use of oral contraceptives and risk of cancer will be discussed in Chapter 4.

Menstrual abnormalities, including intermenstrual bleeding, hypomenorrhoea, and amenorrhoea, may also be observed with use of COCs. Minor complications noted with use of COCs include: nausea, vomiting, headache, leg cramps, mastalgia, bipedal oedema, and chloasma. Leucorrhoea has also been reported and may be due to excessive cervical mucus secretion or to increased risk of monilial infection [23, 222, 225]. Obviously, contraceptive use is not required in pregnancy; however, no adverse effects have been reported with accidental use of COCs in pregnancy [17].

The effect of COCs on lactation has not yet been established [17]. However, COC use is probably associated with a reduction in milk production and alteration of the quality of milk (reduction of protein and fat content) [45, 222]. Moreover, a substantial dose of the COC steroids are ingested by the infant, with effects that are as yet unknown [17, 45, 222].

Because of the unwanted effects associated with use of COCs, there are medical contraindications to their use. These include unexplained vaginal bleeding; circulatory diseases past or present (severe hypertension, valvular heart disease, VTE, MI, or stroke);

family history of DVT/PE; known thrombogenic mutations (i.e. factor V Leiden; prothrombin mutation; protein S, protein C, or antithrombin deficiencies); active systemic lupus erythematosus (SLE); uncontrolled diabetes; cigarette smokers over the age of 35 years; current or prior breast cancer; and active liver or gall bladder disease [23, 45, 222, 223]. Certain antibiotics, anticonvulsants, anti-tuberculosis, and antiretroviral drugs (ARVs) may affect the bioavailability of steroid hormones and, hence, reduce the effectiveness of COCs [17, 45, 223, 224]. Gastrointestinal conditions such as vomiting and diarrhoea may also reduce the efficacy of COCs [223-225].

COCs provide protection against health disorders including pelvic inflammatory disease (PID), endometriosis, fibroids, functional ovarian cysts, benign breast disease, osteopenia, autoimmune disorders of the thyroid, progression of rheumatoid arthritis, and iron deficiency anaemia. They have also been shown to reduce the incidence of endometrial, ovarian, and colorectal cancers. Correction of menstrual abnormalities including regulation of menstrual cycle, reduction of dysmenorrhoea, menorrhagia, premenstrual tension syndrome, and Mittelschmerz (one-sided lower abdominal pain associated with ovulation) syndrome, may also be achieved with use of COCs. They are also used in the treatment of acne, hirsutism, hyperandrogenism, and polycystic ovary syndrome (PCOS) [23, 222, 223]. COCs are highly effective at preventing pregnancy, and the reversibility is prompt (ovulation returns within 3 months of withdrawal of the drug in 90% of cases) [222]. The potential benefits of COCs exceed the risks, in the absence of contraindications.

3.3.1.2 Vaginal ring

The vaginal ring (Nuva Ring) releases ethinyl estradiol 15μg daily, and etonogestrel (a metabolite of desogestrel) 120μg daily. The ring is worn high in the vagina for 3 weeks, it is then removed and after 1 week (after the withdrawal bleeding) a new ring is inserted. Fitting by a health professional is not required. About 10 to 15% of users of the vaginal ring report vaginal-related symptoms such as slight discomfort, a sensation of foreign body, leucorrhoea, vaginitis, or coital problems [23, 45, 222].

3.3.1.3 Transdermal patch

The transdermal patch delivers norelgestromin 150μg, the active metabolite of norgestimate, and ethinyl estradiol 20μg daily for a 7-day period. Three consecutive 7-day patches are applied in a typical cycle, followed by a 7-day patch-free period to allow withdrawal bleeding. Application sites include the buttocks, lower abdomen, upper outer arm, and upper

torso excluding the breast. Contraceptive patch users may have application site reactions [23, 45].

3.3.1.4 Combined injectable contraceptives

Combined injectable contraceptives (CICs) contain the naturally occurring oestrogen, oestradiol, and a progestogen [17]. The preparations are medroxyprogesterone acetate 25mg with oestradiol cypionate 5mg (Cyclofem/Cyclo-provera), and norethisterone enanthate 50mg with oestradiol valerate 5mg (Mesigyna/Norigynon) [225]. Use of "natural" oestrogens in CICs has a favourable impact on lipid metabolism and cardiovascular effects compared to the synthetic oestrogen used in COCs. Each injection should be given on the same date of each month (about every 4 weeks) The contraceptive effect is by inhibition of ovulation [17].

The combined contraceptive vaginal ring, combined contraceptive patch, and combined injectable contraceptives are relatively new, and therefore, there are few data on their long-term effects. Nevertheless, because the vaginal ring and the transdermal patch contain steroids that are used in COCs, rates of serious unwanted effects may be similar and some of the non-contraceptive benefits discussed above may accrue to users of these methods [17, 23].

3.3.2 PROGESTOGEN-ONLY CONTRACEPTIVES

Progestogen-only contraceptives, as the name suggests, contain progestin and are devoid of oestrogen. They are available in the form of oral pills, injectables, and implants.

3.3.2.1 Progestogen-only pills

Progestogen-only pills (POPs) contain a very low dose of a progestin in any one of the following forms: Levonorgestrel 75μg, Norethisterone 350μg, Desogestrel 75μg, Lynestrenol 500μg or Norgestrel 30μg. They are taken daily and preferably at the same time every day (within 2 hours) to avoid pregnancy [23, 222, 225]. They work by making cervical mucus thick and viscous, thereby less permeable to sperm, and the endometrium becomes atrophic, so that implantation is thwarted even if fertilisation does occur. In about 2% of cases ovulation is inhibited [23, 45, 222].

Progestin-only pills have no adverse effect on lactation and hence can be suitably prescribed in lactating women. They also reduce the risk of PID and endometrial cancer [222]. They are effective, and return to fertility is immediate upon discontinuation [225].

POPs are less effective contraceptives than COCs. The efficacy of POPs is affected by certain medications, such as anti-TB drugs (rifampicin and rifabutin), ARVs and anticonvulsants. When the method fails the risk of an ectopic pregnancy is high, although this risk is still lower than in non-contraceptive users [17]. Menstrual abnormalities including irregular bleeding, menorrhagia, and amenorrhoea may be experienced by POP users [23, 45, 222]. Headache, dizziness, and breast tenderness may also be experienced [225].

3.3.2.2 Progestogen-only injectable contraceptives

Progestogen-only injectable contraceptive (POIC) preparations that are commonly used include depot medroxyprogesterone acetate (DMPA), and norethisterone enanthate (NET-EN). Both are administered intramuscularly; DMPA in a dose of 150mg every 3 months or 300mg every 6 months; NET-EN in a dose of 200mg given at 2-monthly intervals [17, 23, 222]. Depot-sub Q provera 104 is also in use; 104mg is administered subcutaneously every 3 months [45, 225]. POICs achieve their contraceptive effect through inhibition of ovulation by suppressing the surge of gonadotropins (mid-cycle LH peak); thickening the cervical mucus thereby preventing sperm penetration; and causing thinning of the endometrium, thus preventing blastocyst implantation [23, 45, 222].

DMPA and NET-EN are highly effective and safe. They also offer health benefits such as reduction in the frequency of sickle-cell crises; improvement of symptoms in women with endometriosis; and reduction of PID, uterine fibroids, and endometrial cancer [23]. The efficacy of DMPA is not affected by use of anti-convulsants, antiretrovirals, or anti-TB drugs (rifampicin or rifabutin), although these may decrease the effectiveness of NET-EN, POPs, and levonorgestrel/etonorgestrel (LNG/ETG) implants [17].

Medical complications that may arise as a result of DMPA use include a reduction in bone mineral density, menstrual irregularity, and amenorrhoea. Following discontinuation of use of POICs, return to fertility is usually delayed (return to baseline fertility may take an average of 10 months). However, the delay is shorter with NET-EN [23, 45, 222].

3.3.2.3 Contraceptive implants

Contraceptive implants are progestin-only delivery systems. They consist of small rods that are usually inserted into a woman's upper arm. Implants currently in use include Jadelle (2 rods containing levonorgestrel 75mg/rod), Implanon (1 rod containing Etonorgestrel 68mg), and Zarin (2 rods containing levonorgestrel 75mg/rod) [225]. Norplant, an implant containing six rods containing levonorgestrel, is no longer in use [45]. Implants are long-term

contraceptives that are effective for 3 to 5 years depending on the type used [225]. They work by thickening the cervical mucus, making it impermeable to sperm; ovulation inhibition; and by causing endometrial atrophy which is unfavourable for implantation [222, 225].

The health benefits accrued from use of contraceptive implants are similar to those of DMPA. In addition, implants offer long-term contraception and there is rapid return to fertility following their discontinuation. Frequent irregular menstrual bleeding, spotting, and amenorrhoea are common among users. Occasionally there is implant expulsion, and there may be difficulty in removal [222, 225].

Progestogen-only contraceptives obviously do not contain oestrogen. As a result, they do not cause many of the unwanted effects associated with COC use. Therefore, they can be used in women who cannot use combined hormonal contraceptives (CHCs) as a result of oestrogen-related contraindications. They may be prescribed to women who have hypertension, fibroids, diabetes, epilepsy, a history of thromboembolism, SLE, or women who are breastfeeding or who are smokers [23, 222, 225]. However, use of progestogen only-contraceptives is associated with a small increase in risk of cardiovascular events in women with pre-existing risk factors [17].

3.4 INTRAUTERINE CONTRACEPTIVE DEVICES (IUDS)

There has been substantial improvement in the design and content of IUDs. The device may be non-medicated (such as the Lippes loop), or medicated by incorporating copper or hormones. Current IUDs in use can be broadly classified into copper-bearing (Cu-IUD), which include NOVA T, Multiload 375, Multiload 250, TCu380S, Copper T200, Gynefix, Copper T380A, and Copper T 220, and Levonorgestrel-releasing IUDs (LNG-IUS) (20μg/24hrs). IUDs are effective for 3 to 10 years [17, 23, 222, 225]. The ideal candidates for the use of IUDs are women in whom combination hormonal contraception is contraindicated [222].

The mechanism by which IUDs exert their contraceptive effect is not clearly understood. Various possible mechanisms have been suggested. Copper induces a non-specific inflammatory reaction in the endometrium along with biochemical changes. These may promote phagocytosis of sperm and impede sperm migration and vitality [23, 222]. The Cu-IUD may interfere with normal development or fertilisation of the ova and may have spermicidal activity [23]. Increased tubal motility, which results in quick migration of the fertilised ovum into the uterine cavity before the endometrium is receptive, and prevention of

implantation by enzymatic interference have also been proposed [222]. LNG-IUS: induces suppression of the endometrium and thus reduces the likelihood of implantation; causes thickening of the cervical mucus, which impedes the ascent of sperm; and alters the uterotubal fluid, which interferes with sperm migration. Insufficient luteal phase activity and anovulation have also been proposed as relevant [23, 222].

IUD insertion is contraindicated in the presence of pelvic infection, dysfunctional uterine bleeding, uterine prolapse, distortion of the shape of the uterine cavity by fibroids or congenital malformation, severe dysmenorrhoea, or past history of ectopic pregnancy [45, 222].

Complications that may arise from use of IUDs include: perforation of the uterus at the time of insertion; pain; abnormal menstrual bleeding, which includes increased menstrual blood loss, prolongation of duration of bleeding and intermenstrual bleeding; pelvic infection (PID); and spontaneous expulsion [23, 45, 222]. However, PID has not been associated with use of LNG-IUS, and the minor unwanted effects of bleeding and cramping are less frequent with these devices, except for irregular bleeding patterns during the first few months of use. In addition, because it releases a potent progestin at the endometrial level, some women experience up to 70% decrease in menstrual flow and 20-25% may become amenorrheic. Dysmenorrhoea tends to improve with use of these devices. Headache, acne, or mastalgia, which could be related to the systemic effects of the progestin, may be experienced with the use of LNG-IUS [23]. In the case of IUD failure, there is a higher risk of ectopic pregnancy, abortion, and preterm delivery. However, because IUDs are highly effective in preventing pregnancy, these complications are less than in non-users. IUDs are not indicated and should not be inserted in pregnancy as they may induce abortion [17, 23].

The LNG-IUS can be used in the treatment of endometrial hyperplasia, adenomyosis, uterine leiomyomas, and endometrial cancer [222]; it has been shown to improve the symptoms of endometriosis [17]. It can also be used as an alternative to hysterectomy for menorrhagia and reduces the hormone-withdrawal symptoms associated with the transition years between end of reproduction and menopause [222]. IUDs provide long-term contraception and, in premenopausal women, there is immediate return of fertility with discontinuation of use [17].

3.5 EMERGENCY CONTRACEPTION

Post-coital or emergency contraception is a therapy used to prevent unwanted pregnancy after unprotected intercourse or after failure to use contraceptive methods appropriately. Methods

used include: combined oral contraceptives (Yuzpe method); levonorgestrel tablets given alone; regular progesterone-only pills, or the copper T 380A IUCD [23, 225]. No fetal adverse effects have been observed when there is failure of emergency contraception.

The hormonal methods prevent pregnancy by delaying or inhibiting ovulation; by slowing down or inhibiting tubal transport of spermatozoa and oocyte; by disrupting the function of the corpus luteum; or by preventing implantation as the endometrium is rendered unfavourable [23, 45, 222, 225]. Users may experience nausea and vomiting especially with oestrogen use. Administration of an anti-emetic may reduce this effect [23, 222].

For women who are eligible for IUD use, a copper-releasing IUD can be inserted within 5 days from the time of unprotected intercourse [17, 45]. The IUD prevents implantation or possibly interferes with sperm function [23]. Anti-progesterone (RU-486 mifepristone and epostane) are also used as emergency contraceptives [45]. Mifepristone inhibits ovulation and prevents implantation, and can be taken up to 17 days after exposure [45, 226].

Emergency contraceptive pills are used for a short duration, and therefore, less clinical impact is expected than with other hormonal contraceptives. Consequently, it can be used by most women. However, emergency contraception should not be used as a regular method. Frequent use may be harmful to women with contraindications to the use of hormonal contraceptives [17].

3.6 STERILISATION (TUBAL OCCLUSION AND VASECTOMY)

3.6.1 TUBAL OCCLUSION

Occlusion of the fallopian tubes in some form is the underlying principle to achieve female sterilisation. This prevents contact between the sperm and ovum. The immediate complications relate to anaesthesia and to the surgical method used in sterilisation; they include pain at the incision site; fever; haematoma; and bladder or bowel injury [222, 225]. Remote complications include chronic pelvic pain; congestive dysmenorrhoea; menstrual abnormalities in the form of menorrhagia, hypomenorrhoea, or irregular periods [222]. Bilateral tubal ligation (BTL) is a highly effective method of contraception. When pregnancy does occur it is more likely to be ectopic [45, 225]. Regret is expressed by 7% of women; this is more common with younger women [45]. Tubal ligation has an inverse association with ovarian cancer and PID [23, 225].

3.6.2 VASECTOMY

Surgical interruption of the vas deferens (vasectomy) is a permanent sterilisation operation done in the male [21, 222]. This blocks the passage of spermatozoa from the testis, and results, therefore, in the absence of sperm in the semen [45, 225]. Vasectomy is considered successful when semen analysis shows azoospermia in two consecutive specimens [21]. With vasectomy sterility is not immediate as it takes up to 3 months or 20 ejaculations for azoospermia to be achieved. This is due to sperm stored beyond the interrupted vas deferens [45]. Additional contraceptive protection is needed for about 2-3 months following vasectomy [222].

Complications may include: scrotal haematoma; wound sepsis, which may lead to scrotal cellulitis or abscess; and sperm granuloma, which is due to an inflammatory reaction to sperm leakage [222]. Vasectomy does not affect hormone levels, spermatogenesis, and rarely affects potency, or sexual performance [21]. Frigidity and impotence, when they occur are mostly psychological [222]. Vasectomy is a highly effective method, the operative technique is simple, and the operation can be done as an outpatient procedure [222]. Vasectomy has a failure rate of less than 1% [21, 45].

There is no medical condition that would absolutely restrict a person's eligibility for sterilisation. Nevertheless, caution should be exercised when there are conditions that may promote intra- and post-operative complications. Male and female sterilisation should be regarded as permanent methods; the success of reversal cannot be guaranteed [17, 45]. Reversal of vasectomy with restoration of vas patency is possible in up to 90% of cases, as demonstrated by the presence of spermatozoa in the ejaculate, but the pregnancy rate is low (30-40%). This difference is attributed to the development of antisperm antibodies following vasectomy [21]. Surgical reversal of tubal ligation results in a pregnancy rate of 45% to 90%, and is associated with a higher risk of ectopic pregnancy. Female sterilisation has a higher failure rate and complication rate, and is more expensive than vasectomy [45].

3.7 BARRIER METHODS

Barrier methods act by preventing sperm deposition in the vagina or by preventing sperm from gaining access to the upper genital tract [222, 225]. The objective is achieved by mechanical devices or by chemical means that produce sperm immobilisation or by combined means [222]. These methods include spermicides, male and female condoms, diaphragms, and cervical caps [17, 23].

3.7.1 SPERMICIDES

Spermicidal methods, available in the form of vaginal jellies, creams, gels, suppositories, vaginal sponge, and foams, in addition to their toxic effect on sperm (they produce sperm immobilisation), act as a mechanical barrier to the entry of sperm into the cervical canal. They may cause irritation to the vagina or vulva [23, 222].

3.7.2 CONDOMS

The male condom or contraceptive sheath is made of latex, polyurethane, or lamb ceca. It serves as a cover for the penis during coitus and prevents the deposition of semen in the vagina [23, 45]. The advantages of the condom are that it provides highly effective and inexpensive contraception as well as protection against sexually transmitted diseases (STDs). Condoms made of latex or polyurethane are impervious to most organisms that cause sexually transmitted diseases. Female condoms are pouches made of polyurethane, which line the vagina and also the external genitalia. The female condom gives protection against STDs and PID and has the additional advantage of being under the control of the woman. However, it is expensive and has a high failure rate [23, 222].

3.7.3 DIAPHRAGM

The diaphragm acts as a mechanical barrier between the vagina and the cervical canal. It is not a popular method as it requires fitting by a health professional, and the necessity for anticipating the need for contraception. There is also the risk of vaginal irritation and urinary tract infection (due to pressure of the rim against the urethra and alterations in the composition of the vaginal flora), and it has a high failure rate [23, 222].

3.7.4 CERVICAL CAP

Cervical caps are small cuplike diaphragms placed over the cervix that are held in place by suction. Tailoring the cap to fit each cervix is difficult, greatly limiting the usefulness of the method. With proper use the efficacy of the cervical cap is similar to that of the diaphragm [23].

Although barrier methods can be used without medical restrictions, due to their low level of effectiveness, they should be used with extra care by women in whom pregnancy is undesirable due to pre-existing medical conditions [17].

3.8 NATURAL CONTRACEPTION

Natural contraception, also known as traditional or folk methods, includes fertility-awareness methods (rhythm or periodic abstinence); the lactation-amenorrhoea method; coitus interruptus; and post-coital douche.

Women are fertile for only a few days of the cycle. Fertility-awareness methods rely on the identification of the fertile period of a cycle and abstaining from sexual intercourse during that period. Accurate prediction or indication of ovulation is essential to the success of this method. The methods used to determine the approximate time of ovulation include:

- recording the pattern of previous menstrual cycles (calendar method);
- noting changes in the basal body temperature (temperature method); and
- noting changes in cervical mucus secretions as affected by menstrual cycle hormonal alterations (cervical mucus/Billings method).

These methods can be used either alone or in combination. In addition, symptoms that may occur just prior to ovulation, such as bloating and vulval swelling can be used as adjuncts in predicting the likely occurrence of ovulation. The disadvantages include:

- difficulty in calculating the safe period;
- compulsory abstinence from the sexual act during certain periods; and
- the need for both a regular menstrual cycle and a well-motivated couple.

In addition, conditions that cause a rise in body temperature, alter cervical mucus or vaginal discharge, or cause irregular vaginal bleeding may affect the use of this method [17, 23, 222].

Lactation provides a natural method of contraception. Suckling leads to increased release of prolactin, which results in a reduction in the release of GnRH, LH, and FSH. This causes anovulation and amenorrhoea [23, 222]. When using this method, the mother must provide breastfeeding as the only form of infant nutrition and must breastfeed through the night; amenorrhoea must be maintained and the method should be practiced as a sole form of birth control for a maximum of 6 months after birth [17, 23, 222].

Coitus interruptus necessitates withdrawal of the penis shortly before ejaculation, resulting in deposition of the semen outside the female genital tract. It has the disadvantage of demanding sufficient self-control by the man and it is possible for pre-ejaculatory fluid containing sperm to flow out before the penis is withdrawn [23, 222].

Plain water, vinegar, and a number of "feminine hygiene" products are used as post-coital douches. The douche flushes the semen out of the vagina, and the additives may possess some spermicidal properties. The method is ineffective and unreliable [23].

Natural family-planning methods have no known health risks or medical contraindications. Return to fertility is immediate. The methods have no cost and are readily available. Natural family planning, like any other client-dependent method, has a low level of effectiveness and, therefore, should be used with care in women with medical conditions that can be worsened by pregnancy [225].

3.9 CONTRACEPTIVE EFFICACY

Contraceptive effectiveness is one of the most important factors in choosing a method [227]. Contraceptive efficacy refers to how well a method would work in a clinical trial (perfect use), while effectiveness refers to how well it works in actual practice (typical use) [228]. Perfect use constitutes correct and consistent use of a method; the failure rate under perfect use is usually lower than under typical use [17, 228]. Contraceptive failure is measured using the pearl index or life-table [227]. The pearl index is the pregnancy rate per hundred woman years (HWY) of use, whereas life-index contraceptive efficacy is the number of women per hundred who become pregnant in the first year of use of a method [223, 228]. Use of the pearl index as a measure of contraceptive efficacy is limited by the fact that the effectiveness of a method improves with longer duration of use; this is because those who are prone to failure do so early [227, 228].

The effectiveness of a contraceptive is dependent on the inherent protection afforded by the method, and how correctly and consistently it is used [17, 228]. It is also affected by the age of the user (fertility declines with age) and frequency of intercourse [228]. User-dependent methods have higher failure rates [17, 227, 228]. The annual failure rate, under perfect use, of COCs is 0.3% and that of LNG-IUS is 0.1%. The failure rate of COCs increases with failure to maintain a regular schedule of use [228].

The most effective contraceptive methods are surgical sterilisation, long-acting progestogen-based contraceptives (DMPA, LNG-IUS, and implants), and IUDs, with first-year failure rates of less than 1% for perfect use. This is because these methods are not user-dependent [223, 227]. The least effective are barrier and natural-contraception methods. The failure rate of lactational amenorrhoea, under perfect use, is 0.4 to 2.0 per 100, and 3.2 per 100 for fertility-awareness methods. Cu-IUDs with $\geq 300 mm^2$ surface area (gross life-table rate of 0.1

to 1.4 per 100) are more effective than Cu-IUDs with <300mm^2 surface area (gross life-table rate of 0.6 to 1.5 per 100). The efficacy of short-acting hormonal contraceptives - injectables, oral contraceptives, transdermal patch, and vaginal ring - is comparable to that of Cu-IUDs with <300mm^2 surface area [227]. Among emergency contraceptives, Cu-T-IUD causes 99% reduction in pregnancies, whereas progestin-only ECPs and COCs have a pregnancy-reduction rate of 89% and 75% respectively [228]. The probability of conceiving per month of exposure (fecundability) is about 20 to 25% in a normal fertile couple; more than 85% achieve conception within one year [23, 45]. The lifetime failure rate of all reversible contraceptives combined for women 15 to 45 years of age is 1.8; with sterilisation, it is 1.3 [228]. A summary of contraceptive efficacy, derived from the publications referenced in section 3.9, is presented in Table 3.1.

Table 3.1: Summary of contraceptive efficacy

Contraceptive method	First year unintended pregnancy rates per 100 women (consistent and correct use)
Combined oral contraceptives	0.3
Progestogen-only pills	0.3
Progestogen-only injectable contraceptives	0.3
Implants	0.05
Copper-bearing IUDs	0.1-1.5
Levonorgestrel-releasing IUDs	0.1
Lactational amenorrhoea method	0.4-2.0
Fertility-awareness methods	3.2
Vasectomy	0.1
Tubal ligation	0.5
No method	85

3.10 CONCLUSION

Other than preventing pregnancy, contraceptives may exert effects that could be beneficial or harmful to the user. The benefits of contraceptive use outweigh the risks; it has been shown that more deaths occur from unintended pregnancies than from contraceptive use [45]. The annual global maternal mortality rate declined by 34% in the period 1990 to 2008 from 400 maternal deaths per 100,000 live births in 1990 to 260 maternal deaths per 100,000 live births in 2008. Avoidance of 1.7 million deaths in this period was attributed to fertility decline, which may partly be due to availability of contraceptives. The bulk of global maternal deaths occur in developing countries. It has been suggested that improved contraceptive availability in developing countries would prevent 94,000 maternal deaths annually [229].

When choosing a contraceptive method, many factors are taken into consideration. These include the efficacy of the contraceptive method, medical eligibility, unwanted effects, availability, acceptability, non-contraceptive benefits, and client's preference. Most couples use contraception to space children or to limit family size, whereas others desire to avoid child bearing because they do not wish to have children, or because of pre-existing illnesses that are likely to worsen with pregnancy. Highly effective long-acting contraceptive methods should be recommended for women in whom unintended pregnancy would be detrimental to their health [17]. Some contraceptives can also be used for treatment of medical conditions and have also been known to accrue health benefits to the users [17]. Individuals should be informed of the available methods, their efficacy, unwanted effects, contraindications, and benefits [17, 45]. Knowledge of non-contraceptive benefits afforded by a method may improve its uptake [226].

An ideal contraceptive would be cheap, safe, highly effective, easy to use, and acceptable to the majority of the population [222]. No one single contraceptive meets this standard. There is ongoing research to identify better and safer contraceptives and to broaden the range of available methods.

The decision to use a contraceptive and the choice of method of contraception is, to a large extent, in the hands of an individual and their clinician, hence modifiable. This is further facilitated by the availability of a wide range of contraceptive methods. Effects on the health of users partly inform these decisions; therefore, availability of information is paramount. Although it is a rare disease, ovarian cancer is highly fatal. The effect of contraceptive use on the risk of ovarian cancer is discussed in Chapter 4.

CHAPTER 4: USE OF CONTRACEPTIVES AND THE RISK OF OVARIAN CANCER

4.1 INTRODUCTION

Ovarian cancer continues to be a devastating disease despite advances in treatment. This is mainly due to the fact that it is detected at an advanced stage (70% diagnosed at stage III) [230] (see Chapter 2 for staging – section 2.1.1). Reproductive factors have been shown to have a substantial influence on the risk of this disease [231]. Of particular interest are contraceptives as these, if causally associated with reduced risk, provide an approach to prevention that is within an individual's control. In addition, because contraceptives are widely used, even a small variation in risk would have far reaching consequences for public health.

From Chapter 3, it is clear that there are many methods of contraception available, each with different characteristics that may impact on choice and adherence. It is also notable that the pathogenesis of ovarian cancer has not yet been established, although many theories have been suggested (see Chapter 2 – section 2.4). In this chapter, issues regarding the relationship between contraceptive use and the risk of ovarian cancer will be discussed. First, tumour initiation and normal female endocrine function will be discussed briefly. Knowledge of these is important in understanding possible ways through which contraceptives may affect ovarian carcinogenesis. A summary of epidemiologic studies assessing associations between contraceptive use and ovarian cancer will then be presented followed by a discussion of the proposed biologic mechanisms that underlie these findings. Finally, an overview of the association between contraceptive use and the risk of other cancers will also be presented.

4.2 HOW DOES A NORMAL CELL BECOME NEOPLASTIC?

The human body replaces one million cells every second [58]. The rate of production is dependent on the cell cycle. All replicating cells, whether normal or neoplastic go through a series of phases termed the cell cycle. In S-phase, DNA synthesis occurs; cells in G_2 (pre-mitotic phase) have double the DNA content; in M-phase (mitosis), the cell divides into 2 new cells. Protein synthesis, RNA synthesis, and DNA repair occur in G_1 (resting phase). From G_1, cells either progress through the cell cycle or enter G_0 (quiescent phase) [45]. The cell generation time is the duration taken to go through the cell cycle. Neoplastic tissues do not have shorter generation times, but a high number of cells actively replicating (higher

growth fraction). On the other hand, a large proportion of cells in normal tissues are in G_0 [23, 45].

Several theories of carcinogenesis have been proposed including gene mutations, familial predisposition, non-genotoxic effects, cell selection, and effect of microenvironment and morphostats [232]. The human genome faces many assaults in a day, more so for cells undergoing rapid proliferation [20, 58]. As cells divide, DNA copying errors may occur that may lead to genetic or epigenetic changes necessary for neoplastic transformation [233]. For most cancers more than one mutation is required for cancer to occur [20, 234]. These critical mutations occur in genes responsible for cell-cycle control and maintenance of the quality of the DNA genome [234]. Fortunately, the body has mechanisms of protecting itself against cancer. These include DNA repair, cell cycle arrest, apoptosis, immune mechanisms [20, 58], and morphostats [232].

A potential tumour precursor cell has to surmount the body's defence system for a tumour to develop [235]. An incipient tumour cell achieves this by acquiring a sufficient number of mutations to drive the tumourigenesis process [58]. This process enables the tumour cells to acquire characteristics that distinguish them from normal cells, which include:

- autonomy from normal proliferative and antiproliferative signals;
- avoidance of apoptosis;
- ability to divide indefinitely; and
- ability to induce angiogenesis and deranged metabolism [20, 58, 235].

In addition, the body's immune system, which provides protection against cancer as evidenced by higher incidence of cancer in patients with impaired immunity [20] can, paradoxically, be manipulated by tumour cells to their advantage, such that it promotes instead of preventing growth [58]. Prevention of cancer can be achieved by modulating the action of factors that are involved in the process of carcinogenesis or in the sustenance of the cancer cells [58].

4.3 NORMAL FEMALE REPRODUCTIVE ENDOCRINE FUNCTION

Use of hormones has been shown to be associated with risk of developing some types of cancer [236]. Response by cells to a particular hormone is dependent on the presence of receptors - often specific to the hormone - on the target cell [20, 21]. Hormones can modify the risk of cancer in organs in which they control growth and development or function. This

has been demonstrated in breast, prostate, endometrial, ovarian, testicular, and thyroid cancer [233, 237]. A reduction in risk of these cancers is established or possible by regulation of the action or levels of the specific hormones responsible [233].

Hormones are thought to increase the risk of cancer, at least in part, by increasing the proliferation rate of cells in the organs under their influence. This increases the probability of occurrence of genetic errors that lead to neoplasia and of the affected cells undergoing replication before DNA repair [237, 238]. Higher proliferation rates also promote the growth of tumours [237].

Endogenous reproductive hormones are produced at different levels depending on a woman's phase of life. A woman's reproductive life begins at menarche, which occurs at an average age of 12 years [21] and ends at menopause (about 51.5 years). If menopause occurs prior to 40 years of age, it is considered premature ovarian failure; this condition is characterised by raised levels of FSH. Iatrogenic causes such as surgery, radiotherapy, or chemotherapy may also result in early menopause [45]. Menarche can be defined as the first menstrual period in life; menopause is marked by the last menstrual period (menopause is sometimes defined as 12 months following the last menstrual period), and is thought to result from loss of ovarian function [21]. At menarche, there are fewer than 300,000 ova [23] of which about 400 mature and are discharged from the ovary (ovulation), with the majority undergoing atresia [20]. It is important to note that no new ova are formed after birth [23]. By the peri-menopausal period, the number of follicles has been reduced substantially, resulting in a reduction in oestrogen production. When oestrogen levels drop to very low levels, the negative feedback inhibiting gonadotropin production from the pituitary gland is removed; this leads to high levels of FSH and LH in the menopausal period [20].

Normal female reproductive endocrine function is controlled by the hypothalamo-pituitary-ovarian axis. GnRH from the hypothalamus stimulates the production of gonadotropins by the anterior pituitary gland [20]. The gonadotropins (FSH and LH) produced by the anterior pituitary gland control ovarian steroid hormone production. Oestrogens are produced through the combined action of LH and FSH. LH acts on the theca cells to stimulate androgen production which diffuses to the granulosa cells. FSH activates the aromatase enzyme present in the granulosa cells, and this, in turn, converts androgens to oestrogens. Androgen production is stimulated by LH, whereas that of progesterone is by both FSH and LH. In the ovary, receptors for LH are found on cells that produce progesterone and androgens, which

are the corpus luteum, follicular theca cells, and stromal cells involved in hormone production. Granulosa cells are the only cells that have FSH receptors [21]. Progesterone and oestrogen exert a negative feedback effect on the anterior pituitary gland and, to some extent, the hypothalamus resulting in a decrease in FSH and LH production [20].

The principal progestin is progesterone, the other being 7-α hydroxyprogesterone, whereas there are 3 types of oestrogens: β-oestradiol, oestrone, and oestriol. Among the oestrogens, oestradiol has the highest potency: 12 times that of oestrone and 80 times that of oestriol. The ovaries produce mainly β-oestradiol and a small amount of oestrone. Oestrone is largely formed from conversion of androgens in the peripheral tissues. Oestriol is derived from oestradiol and oestrone, mostly in the liver. Large amounts of oestriol are also produced by the placenta during pregnancy. In premenopausal women, progesterone is mainly produced following ovulation from the corpus luteum (in the secretory phase of the menstrual cycle). High levels of progesterone are also produced in pregnancy [20]. Androgens are produced mainly by the ovaries and adrenal glands in premenopausal women. After menopause, the elevated gonadotropin levels stimulate ovarian androgen production from the secondary interstitial cells derived from cells of follicles that have undergone atresia and from hilar cells [239]. Whereas there is one form of androgen receptor (AR), there are 2 isoforms of oestrogen nuclear receptors (ERα & ERβ) and G-protein-coupled membrane receptors, and 2 of progesterone receptors (PRA and PRB) which serve different functions [45].

At the beginning of pregnancy the corpus luteum produces oestrogen, progesterone, and human chorionic gonadotropin (hCG). Later, this function is taken over by the placenta. Most of these hormones enter the maternal circulation. In pregnancy, a woman's circulating levels of progesterone increase from 25ng/ml at the end of the luteal phase to 150ng/ml at the end of pregnancy [21]. Oestriol ("pregnancy oestrogen") is also produced in large quantities during pregnancy; however, oestradiol production is also substantially increased [45]. In late pregnancy, daily placental oestrogen production is approximately 30 times the non-pregnant level. The high levels of oestrogen and progesterone inhibit the production of gonadotropins from the pituitary gland. Similarly during lactation, ovulation is inhibited and there is suppression of FSH and LH production [20].

Prior to menopause, oestradiol is produced mainly by the ovaries and, to a lesser extent, by peripheral conversion, mainly in adipose tissue, of adrenal androgens to oestrogen. During this period of life, circulating oestrogen levels are under the control of the hypothalamo-

pituitary-ovarian axis feedback loop. In post-menopausal women, oestradiol and progesterone production in the ovaries ceases and the main source of oestrogen becomes peripheral conversion, by aromatisation, of adrenal androgens to oestrone, some of which is then converted to the more potent oestradiol. Plasma oestradiol levels drop to about 10% of the pre-menopausal levels and progesterone levels are also greatly reduced. Oestrogen production at this stage in life is unregulated – and largely dependent on adipose tissue: in pre-menopausal women, high BMI (>30kg/m^2) causes anovulation and a fall in sex hormone-binding globulin (SHBG) levels, resulting in higher oestradiol levels in early follicular phase. However, due to feedback control, oestradiol levels remain constant, but the progesterone levels are affected due to anovulation. In post-menopausal women, higher oestrogen levels are observed in obese women due to increased peripheral conversion of androgens to oestrogen and the lack of feedback control [237].

Steroid hormones are transported in circulation attached to proteins, serum albumin and SHBG, leaving less than 10% free. The free hormones are considered active, whereas protein-bound hormones are presumed to be biologically inactive as their ability to diffuse across capillaries to reach the target cells is limited [20, 58].

The main source of exogenous hormones for women are hormone-containing contraceptives and, to a lesser extent, pharmaceutical PMH use [236]. Women can choose to use hormone-containing contraceptives during their reproductive life and PMH after menopause. Hormonal contraceptives contain progesterone alone or in combination with oestrogen. Cancers arising as a result of use of hormonal contraceptives are, essentially, related to the action of the constituents. Contraceptives that do not contain hormones may affect the risk of cancer through other mechanisms related to the pathogenesis of the specific tumour.

4.4 EPIDEMIOLOGIC STUDIES ON THE ASSOCIATION OF OVARIAN CANCER AND USE OF CONTRACEPTIVES

4.4.1 INTRODUCTION

Oral contraceptives have been shown to be inversely associated with ovarian cancer [7, 12, 14]: ever-use of oral contraceptives is associated with a 30-40% lower risk than never-use [7, 13]. If considered causal - as seems reasonable given both the consistency of the epidemiologic data and the plausibility of the likely mechanisms – then we can say that protection is greater with longer duration of use and is about 20% after 5 years of use, increasing to 50% or more after 15 years of use [14, 240]. This protective effect declines with

time since last use and is no longer present 20 years after cessation [7, 240]. A similar beneficial effect is seen in *BRCA* mutation carriers and in women with a family history of ovarian cancer [240], albeit from a higher baseline risk. Other factors known to be associated with a lower risk of ovarian cancer include parity, breastfeeding, tubal ligation, and hysterectomy [15].

The association between use of other types of contraceptives and the risk of ovarian cancer has not been established, and is the focus of this thesis (Chapter 1 provided the research questions, hypotheses, and objectives for the thesis). Here I present a review of recent epidemiologic studies addressing the relationship between the risk of ovarian cancer and the use of IUDs, long-acting progestogen-based contraceptives, and spousal/partner vasectomy.

4.4.2 METHODS

Relevant epidemiologic studies were identified through a search of the MEDLINE, EMBASE and CINAHL databases using the key words ('ovarian cancer'/'ovarian tumour') and ('injectable contraceptive agent'/ 'medroxyprogesterone acetate' /'contraceptive implant') and ('intrauterine contraceptive device'/intrauterine contracept*') and 'vasectomy' and 'cancer risk'. All studies published in English to December, 2014 were included (See Appendix 1 for search strategy). Additional papers were found by checking reference lists.

4.4.3 LONG-ACTING PROGESTOGEN-BASED CONTRACEPTIVES

The association between progestogen-based, long-acting contraceptives and the risk of ovarian cancer, of which DMPA has been most studied, has not been established. In a study in Shanghai involving 229 women with ovarian cancer and a similar number of controls, there was no statistically significant association between DMPA and ovarian cancer: compared to never-use, ever-use of DMPA was associated with an OR of 2.8 (95% CI = 0.9-8.5). There was no relationship between duration of DMPA use and the risk of ovarian cancer. However, only a small number of participants had used DMPA (24 cases and 6 controls). In addition, in this study, contrary to what is otherwise well established, an inverse association between oral contraceptives and ovarian cancer was not found (OR = 1.8; 95% CI = 0.8-4.1, confined to short duration users [<1 year]) [11].

The WHO Collaborative Study of Neoplasia and Steroid Contraceptives, conducted in Mexico and Thailand, found no association between DMPA use and ovarian cancer. There was also no relationship between ovarian cancer and patterns of use of DMPA (duration of use; time since first use). Ever-use of DMPA was associated with an RR of 1.07 (95 % CI =

0.6-1.8). Although there was a difference in risk between histologically borderline and malignant tumours and among histological subtypes, the differences were not statistically significant. However, the study had low statistical power to detect an association between DMPA use and epithelial ovarian cancer (23% chance of detecting a 25% risk reduction of EOC, at a significance level of 0.05, in ever-users of DMPA and much lower power to detect association with duration of use). In addition, the association between DMPA use and ovarian cancer risk in nulliparous women could not be assessed, as DMPA is not prescribed to nulliparous women in Thailand [10].

Two recent studies have found DMPA to be inversely associated with ovarian cancer, with evidence of a dose-response relationship with longer duration of use. In a large hospital-based case-control study involving South African black women, compared to never-use of hormonal contraceptives, ever-use of injectable contraceptives was found to be inversely associated with the risk of ovarian cancer (OR = 0.35; 95% CI = 0.17–0.71; P = 0.004). Exclusive use of injectable contraceptives for ≥5 years was also associated with a statistically significant lower risk (OR = 0.07; 95% CI = 0.01–0.49; P = 0.008); however, it is of particular note that the association with long duration of use was based on only one case. In addition, in this study, injectable contraceptives were assumed to be progesterone-only preparations; however, use of combined injectable contraceptives in this group of women cannot be ruled out [12].

A more recent hospital-based case-control study carried out in Thailand found a statistically significant lower risk in ever-users compared to never-users of DMPA (OR = 0.61; 95% CI = 0.44–0.85; P = 0.002). An inverse trend in risk was observed with longer duration of use, with a statistically significant lower risk being observed after more than 3 years of use (OR = 0.17; 95% CI = 0.07-0.39; P <0.001). However, the histology of cases was not reviewed by a single pathologist and, therefore, there is the risk of inter-pathologist variation. So far, this is the largest case-control study done to assess the association between DMPA use and risk of ovarian cancer [13].

4.4.4 INTRAUTERINE CONTRACEPTIVE DEVICES (IUDS)

Studies have produced conflicting results regarding the use of IUDs and ovarian cancer. In the Shanghai case-control study, compared to never-use, ever-use of IUD was associated with a possibly lower risk of ovarian cancer (OR = 0.5; 95% CI = 0.2-1.1) [11].

A prospective cohort study, also done in Shanghai, reported no association between risk of ovarian cancer and onset of use of IUDs at ≥30 years of age (HR =0.90; 95% CI = 0.51 – 1.62). Overall, no association was found between use of any contraceptive method and ovarian cancer, including oral contraceptive use and tubal ligation, (HR = 1.10; 95% CI = 0.66–1.82 and HR =1.17; 95% CI = 0.62–2.20, respectively). This study had a small number of cases and the sample size and power were probably inadequate to assess the association between contraceptive use and ovarian cancer. In addition, information was not available on the type of IUDs used [241].

In contrast, the US Nurses' Health Study reported a statistically significantly higher risk of ovarian cancer for ever-users of IUDs compared with never-users (RR = 1.76, 95% CI = 1.08-2.85), with the association being stronger for serous and endometrioid histological subtypes: RR = 2.17 and RR = 2.40 respectively (95% confidence intervals for these relative risks were not provided). In that study, familial predisposition to ovarian cancer was not considered in the analysis and the relationship between risk and duration of use was not reported. In addition, the IUDs used by participants in this study were mainly the copper-bearing type and may not reflect the association with the newer levonorgesterel-releasing IUDs (LNG-IUS) [7].

Ness et al. observed a lower risk of ovarian cancer with IUD use (OR = 0.77; 95% CI = 0.61−0.99). In that study, IUDs were inversely associated with ovarian cancer among those with short duration of use (OR = 0.53; 95% CI = 0.39-0.72 for use for ≤4 years), but there was statistically non-significant association with prolonged use (OR = 1.11; 95% CI = 0.63-1.96 and OR = 1.40; 95% CI = 0.82-2.39, for use for 5-9 years and ≥10 years respectively). It is instructive to note that only a small number of participants (n = 14) had used the progestin-containing IUD which is thought to be inversely associated with risk. In this population-based case-control study, all the contraceptive methods assessed were found to be inversely associated with ovarian cancer [14].

4.4.5 VASECTOMY

There have been few studies of the association between partner vasectomy and ovarian cancer. The US Nurses' Health Study, a prospective cohort study, found no association between spousal vasectomy and ovarian cancer (RR = 0.87; 95% CI = 0.63-1.19) [7], whereas a population-based case-control study found an inverse association of borderline statistical significance with vasectomy (OR = 0.7; 95% CI = 0.61-0.99) [14].

A summary of all the above studies assessing the relationship between spousal vasectomy, use of IUDs, DMPA, and the risk of ovarian cancer is presented in Table 4.1.

Table 4.1: Summary of epidemiologic studies assessing the association of spousal vasectomy, use of IUDs, and DMPA and the risk of ovarian cancer

Author (Year)	*Study Period*	*Study Design and Area*	*Study population*	*Results*		*Adjusted for :*	*Comments:*
				Ever Vs Never use	*Patterns of Use*		
Wilailak et al.[13] (2012)	2006 to 2008	Hospital-based case-control study (12 Hospitals across Thailand)	Cases: 330; 20 -70 years old; with EOC Controls: 982 age matched patients Case-control ratio: 1:3	DMPA: OR=0.61 (95% CI=0.44-0.85; P=0.002)	DMPA: use for >3 years: OR=0.17 (95% CI=0.07-0.39; P<0.001)	Breastfeeding, combined OCs, parity, family history of gynaecological cancer	The largest case-control study assessing the association between DMPA use and ovarian cancer
Urban et al. [12] (2012)	1995 to 2006	Hospital-based case-control study (Johannesburg, South Africa)	Cases: 182; 18 -79 years old; with ovarian cancer Controls: 1,492 women with cancer types that are not associated with hormonal contraceptives Case-control ratio: 1:8	Injectables: OR=0.35 (0.17–0.71; P = 0.004)	Injectables use for >5 years: OR=0.07 (95% CI=0.01–0.49; P = 0.008)	Age, year of diagnosis, education, smoking, alcohol, parity, age at first birth, & number of sexual partners	High prevalence of use of injectable contraceptives (26%). Low power to assess associations by type of contraceptive used. OCs assumed to be combined OCs, and Injectables to be progesterone-only preparations
Ness et al. [14] (2011)	2003 to 2008	Population-based case-control study (Western Pennsylvania, Eastern Ohio, & Western New York State)	Cases: 902; ≥ 25 years old; with 1^0 epithelial ovarian, fallopian tube, or peritoneal cancer. Controls: 1,800 frequency matched by age & telephone exchange Case-control ratio of 1:2	IUDs: OR=0.75 (95% CI =0.59−0.95) Vasectomy: OR=0.77 (95% CI= 0.61−0.99)	IUD-duration of use: ORs 0.53 for ≤4 years; 1.11 for 5−9 years; 1.40 for ≥10 years	Age, parity, race, infertility, family history of ovarian cancer, and use of other contraceptives	Borderline tumours included Histological slides reviewed by study pathologist. Use of any type of contraceptive was inversely associated with ovarian cancer.
Dorjgochoo et al. [241] (2009)	1996 to 2006 Recruitment:1997-2000 Median follow-up: 7.5 years	Population-based prospective cohort study (Shanghai, China)	66, 661 women; 40-70 years old at recruitment 183 diagnosed with ovarian cancer during follow up.	IUD: HR= 1.03 (95% CI= 0.62-1.73)	IUD: Years of use: < 14 HR =1.13 (95% CI=0.64-2.02); ≥14 HR=0.93 (95% CI=0.51-1.70) IUD: Age- 1^{st} use: <30 HR= 1.23 (95% CI=0.68-2.24); ≥30 HR=0.90 (95% CI=0.51-1.62)	Education, age at menarche, parity, BMI, regular exercise, smoking, menopausal status, & family history of cancer.	Not affected by PMH use (only 3% of participants had used PMH) Histological slides reviewed by study pathologist. Low statistical power Lack of information on specific type of contraceptive used

Author (Year)	***Study Period***	***Study Design and Area***	***Study population***	***Results***		***Adjusted for***	***Comments***
				Ever Vs Never use	*Patterns of use*		
Tworoger et al. (2007) [7] (US Nurses' Health Study)	1976 to 2004 (28 Years Follow up)	Prospective cohort study (USA)	107,900 US married, female registered nurses; 30-55 years old. 612 diagnosed with invasive EOC during follow up	IUD: RR=1.76 (95% CI= 1.08-2.85) Vasectomy: RR=0.87 (95% CI= 0.63-1.19)	Not reported	Age, current BMI, BMI at 18 years, parity, HRT use, smoking, duration of OC use, and age at menarche & menopause	The longest prospective cohort study of incident ovarian cancer, published to date. Repeated measure of variables of interest Did not control for family history of cancer Histology reports reviewed by study pathologist. Included tumours of borderline malignancy
Stanford & Thomas. (1991) [10] (The WHO Collaborative Study of Neoplasia and Steroid Contraceptives)	1979 to 1988	Hospital-based case-control study (Mexico & Thailand)	Cases: 224; born after 1925; with EOC. Controls: 1,781; matched on age, hospital and year of interview Case-control ratio: 1:8	DMPA: RR=1.07 (95% CI= 0.6-1.8).	DMPA: Use for ≥ 5 years: RR=1.09 (95% CI = 0.4 – 3.2) Age in years at diagnosis: ≤40, RR=1.35 (95% CI=0.7-2.6); > 40, RR=0.82 (95% CI=0.3- 1.9)	Parity and oral contraceptive use.	Association between DMPA use and ovarian cancer risk in nulliparous women could not be evaluated. Low power to detect association between ovarian cancer and DMPA. Included tumours of borderline malignancy
Shu et al. [11] (1989)	1984 to 1986	Population-based case-control study (Shanghai China)	Cases: 229; 18 to 70 years old; with ovarian cancer. Controls: 229; matched on age Case-control ratio: 1:1	DMPA: OR= 2.8; (95% CI=0.9-8.5) IUDs: OR= 0.5 (95% CI=0.2-1.1)	DMPA: No relationship with duration of use IUD: Not reported	Education, parity, ovarian cyst, and age at menarche	No association with ever-use of OCs (OR=1.8; 95% CI= 0.8-4.1). Low statistical power.

4.4.6 DISCUSSION

The findings of the earlier studies of DMPA use and ovarian cancer risk are surprising. This is because, as will be discussed later, the biologic effects of DMPA could be expected to be protective. However, the low number of participants in these studies and the fact that DMPA had been used only for a short time make it hard to assess possible associations with confidence [13].

Differences in associations between long-term and short-term use of IUDs were reported. The higher risk with longer duration of use has been attributed to the fact that IUDs require replacement every 5 to 10 years; thus, longer use means more replacements. This increases the risk of upper-genital-tract infection. In contrast, the lower risk associated with short-term use may be explained by the spermicidal effect of IUDs, thus reducing local inflammation [14], or use of the newer levonorgesterel-releasing IUDs (LNG-IUS), which may be associated with lower risk of ovarian cancer.

The relationship between spousal vasectomy and the risk of ovarian cancer is the least studied. Available evidence points towards no association or an inverse association.

It has been suggested that the inverse association with ovarian cancer observed with use of contraception in general can be explained by the fact that infertile women tend not to use contraceptives and are at a higher risk of ovarian cancer; therefore, the higher risk in non-users could be confounded by infertility. On the other hand, it could be that contraceptives have a relationship with ovarian carcinogenesis that is yet to be understood [14].

The above studies had several limitations. First, there was the risk of misclassification of controls as they were not tested for the presence of asymptomatic ovarian cancer; however, ovarian cancer is uncommon and, therefore, the prevalence of undiagnosed ovarian cancer in controls would be expected to be low. Second, in most of these studies, histology was not reviewed by a single pathologist and this poses the risk of inter-pathologist variation. There was also the possibility of recall bias, especially in relation to duration of use of specific contraceptives. As women often use more than one type of contraceptive, it is also difficult to disaggregate possible differences associated with the use of different contraceptives. In addition, most of the studies had insufficient numbers of participants, and did not report associations with long-term use. Finally, no studies have been undertaken on the use of progestin-containing IUDs.

In conclusion, oral contraceptives are well known to be strongly inversely associated with, and probably (see below) causally protective against, ovarian cancer. The association with use of other contraceptives on the risk of ovarian cancer still merits attention.

4.5 BIOLOGICAL EXPLANATION OF THE EPIDEMIOLOGIC FINDINGS

4.5.1 INTRODUCTION

Epidemiologic studies provide information on possible causes of disease [242]. However, other than epidemiologic evidence, knowledge of the mechanism of effect is also required for a causal association to be established between an agent and cancer [58], which helps in fulfilling Bradford Hill's causal criterion of 'biological plausibility' [144]. OCs have been shown to be inversely associated with ovarian cancer. Several hypotheses to explain this association have been suggested, these include: inhibition of ovulation; suppression of endogenous gonadotropin, androgen, and oestrogen production; and increased levels of circulatory progesterone. The suggested mechanisms of protection are in-line with the proposed pathogenesis and established risk factors of ovarian cancer and the biologic effects of OCs. An understanding of the mechanisms of protection conferred by OCs may shed light on the possible ways in which other contraceptives may work in this context and also help in developing other preventive strategies. Herein, the mechanisms by which OCs may provide protection against ovarian cancer will be discussed, followed by a discussion on the probable effects, and the potentially beneficial – as well as risk-increasing - mechanisms in ovarian carcinogenesis of long-acting progestogen-only contraceptives, IUDs, and vasectomy.

4.5.2 ORAL CONTRACEPTIVES

4.5.2.1 Inhibition of Ovulation

Combined oral contraceptives (combined OCs) are thought to prevent ovarian cancer by inhibiting ovulation [243]. Ovarian surface epithelial (OSE) cells adjacent to the site of ovulation are exposed to inflammatory mediators and reactive oxidants that can cause DNA damage [235, 244]. One source of possibly damaging free radicals is thought to be leukocytes that infiltrate developing follicles [244]. Following ovulation, epithelial cells replicate to cover the defect on the ovarian surface [242, 244]. The higher proliferative rate decreases the chance of DNA repair or apoptosis of cells that have sustained genomic damage [245]. In addition, formation of the corpus luteum is also associated with angiogenesis [55]. Following ovulation, DNA repair or efficient removal of incipient tumour cells or both are essential in avoiding cancer. Therefore, defects in genes involved in these processes, such as *BRCA 1/2* and *p53*, increase the probability of ovarian cancer development [244]. Proliferation of a cell

that has sustained DNA damage is thought to be the initial event in ovarian carcinogenesis [55, 235, 244]. Lifetime duration of ovulation has been shown to be associated with the risk of ovarian cancer at a mean of 6% per year, with the 20-29 year age group being at the highest risk, namely 20% per year of ovulation [246]. Inhibition of ovulation therefore, reduces the chance of genetic damage to ovarian epithelial cells [243].

It has been suggested that ovarian cancer arises from cortical inclusion cysts (CICs), [53, 247] and this is supported by the observation that early ovarian cancer is confined to the organ without surface involvement [239]. CICs are thought to form during the ovulatory-repair process [53, 247]. In support of this is a study in which the number of CICs was positively associated with ovulatory age: 25-34 year-old women had a mean of 2.6 GICs, whereas those of 55 years and above had a mean of 4.5 GICs (Spearman's rho = 0.2; P = 0.06). There were also fewer CICs in ever-users of oral contraceptives. However, this study lacked power to assess weak associations and did not include young women [247]. Extra-ovarian sources of CICs have also been proposed [239].

Ovulation includes inflammatory processes, with leukocyte infiltration, and release of inflammatory mediators [55]. Among the theories explaining the pathogenesis of ovarian cancer is an inflammatory theory, which is supported by the observed lower risk associated with prolonged use of anti-inflammatory drugs (i.e., NSAIDS, including ASA) [235]. Conversely, asbestos and talc (which induce inflammatory responses) are thought to increase the risk of ovarian cancer [235] although the relationship between talc and ovarian cancer is inconclusive [246].

Consistent with the incessant-ovulation hypothesis are the plausible protective effects of OC use, breastfeeding, and parity, conditions in which ovulation is suppressed [235, 239, 244, 245]. However, several factors serve to undermine the incessant-ovulation theory. Most importantly, the strengths of the inverse association between OC use and parity and ovarian cancer far outweigh that which can be attributed to inhibition of ovulation alone [55, 237, 239, 248]. Women ovulate for an average of 20 years in their lifetime. Each full-term pregnancy inhibits ovulation for about 1 year, which reduces lifetime ovulations by 5-6%. It has been shown that, in parous women, for each additional pregnancy, the reduction of risk of ovarian cancer is 14-16%. Similarly, OCs are inversely associated with risk at 9% per year of use, which is greater than the 5-6% protection expected from inhibition of ovulation [53]. Secondly, women with polycystic ovary syndrome (PCOS), a condition characterised by

decreased ovulation or anovulatory cycles, have an increased risk of ovarian cancer [53, 55, 235, 245, 249]. Inhibition of ovulation alone also does not explain the more marked inverse association with twin pregnancies, given that twin pregnancies are associated with increased ovulation [239], nor the inverse association with tubal ligation and hysterectomy [249]. Furthermore, normal and malignant ovarian surface epithelial cells have receptors for FSH, LH, GnRH, oestrogens, progesterone, and androgens, suggesting a role for these hormones in ovarian carcinogenesis [243, 246]. In view of the weaknesses of the incessant-ovulation hypothesis, additional complementary mechanisms, mainly hormonal, have been proposed to account for the inverse association between OC use and ovarian cancer [53, 239, 245, 248].

4.5.2.2 Extra-Ovarian Origin Theory

The tubal fimbriae lie in close spatial proximity to the ovaries and may, therefore, be exposed to substances produced during ovulation [250]. The most prevalent and highly fatal, serous type of ovarian cancer, is now postulated to be of tubal origin [51]. A relevant question is therefore: what is the effect of ovulation on the tubal cells?

In a study of mice and baboons, ovulation was found to increase both macrophage infiltration into the fallopian tube, and DNA damage of tubal epithelial cells (TEC). Macrophages produce substances that promote inflammation. No increase in proliferation of TEC was noted. However, these effects were assessed at 12 hours and 16 hours after injection of hCG which does not rule out the possibility of proliferation occurring later in the post-ovulatory period. In addition, exposure of cells to hydrogen peroxide led to an increase in DNA damage [34]. Hydrogen peroxide is used to mimic the oxidative stress caused by ovulation. On the other hand, menopausal levels of gonadotropins (FSH and LH), and oestradiol (10nM) did not have genotoxic or proliferative effects on TEC in spite of the presence of receptors for these hormones on these cells (FSHR, LHR, and ERα) [250].

In a study of *BRCA* mutation carriers, p53 signatures were statistically significantly associated with low parity and older age at first childbirth. Compared to nulliparous women, women with a parity of 3 or more had an OR of 0.2 (95% CI = 0.04-0.9; P-trend = 0.02). After adjusting for age and parity, the OR for women with a BMI $\geq$30kg/M^2 compared to those with a BMI of < 25kg/M^2 was 0.3 (95% CI = 0.1 - 1.3). This study was limited by a small sample size and missing data. Furthermore, no association was found between p53 signatures and OC use, or age [251]. These findings add some support to the incessant-ovulation hypothesis.

If ovarian cancer cells arise from extra-ovarian sites, why is the ovary the most affected organ? In a case-control study, Pearce et al. found a positive association between low-grade serous carcinoma (LGSC) and a history of endometriosis (OR = 2.11; 95% CI = 1.39-3.20), which is unusual: endometriosis is associated with higher risk of ovarian cancer, specifically the endometrioid and clear-cell histologic subtypes. To explain this, they hypothesised that the LGSC could arise from endosalpingiosis which is usually asymptomatic, and therefore, difficult to study using a case-control design. They further postulated that the host's susceptibility to implants of endometrial tissue may also be responsible for an increased risk of endosalpingiosis, which is as a result of implantation of cells from the fallopian tube, both being of Müllerian origin [252]. Therefore, it is possible that the ovaries of women who develop ovarian cancer provide a suitable microenvironment for the development of precancerous cells [128]. Indeed, it has been shown that local microenvironment can influence the transformation of normal cells to malignant cells. Conversely, the microenvironment can also cause malignant cells to differentiate into normal cells [253]. It has also been argued that secondary sites of cancer metastasis do not occur by chance. Metastatic cancer cells develop only in sites that provide a suitable environment – the "seed and soil" hypothesis of metastatic spread [58].

4.5.2.3 Gonadotropins

Excessive gonadotropin levels are thought to play a facilitating role in the development of ovarian cancer [245, 249]. In this case, gonadotropins, acting directly or via increased production of oestrogen, stimulate malignant transformation of ovarian epithelial cells [53, 55, 239, 245]. Oral contraceptives cause a decrease in both peak and basal levels of gonadotropins; this has been proposed as one protective mechanism against ovarian cancer [53]. FSHR and LHR are expressed by theca and granulosa cells in developing follicles [245] and by normal and malignant OSE [245, 246].

Hormones are thought to cause cancer by promoting cell proliferation, thereby increasing the chance of genetic errors that lead to neoplasia. Syed et al demonstrated that FSH and LH, at doses equivalent to the normal plasma levels in women (20-200mIU/ml), were equally potent in stimulating growth of normal and malignant OSE cells. The effect was mediated via specific hormone receptors and was dose-dependent [238]. OCs, therefore, by inhibiting the production of gonadotropins, decrease stimulation of proliferation in the ovary, thus reducing risk of malignant transformation of ovarian epithelial cells [243].

Increased incidence of ovarian cancer has been demonstrated in castrated rats whose ovaries were auto-transplanted to the spleen. Due to portal vein drainage, the oestrogens are destroyed by the liver before they reach the systemic circulation and, therefore, negative feedback of pituitary gonadotropin secretion is eliminated, resulting in high levels of gonadotropins. However, it is of note that the tumours were of granulosa cell types, and therefore of thecal, not epithelial origin [53, 233, 245].

Furthermore, in a study that assessed the levels of gonadotropins in peritoneal fluid, compared to controls (women with functional or benign ovarian cysts), patients with ovarian cancer and those with borderline ovarian tumours had higher concentrations of LH in the peritoneal fluid ($P = 0.005$ and $P = 0.007$, respectively). Similarly, compared with functional and benign ovarian cysts, the level of LH in cyst fluid was higher in borderline and malignant cysts ($P = 0.02$ and $P = 0.008$, respectively) [254]. However, in another study, participants with ovarian cancer were found to have lower FSH levels than those without ovarian cancer ($P = 0.04$); no significant difference was found in LH levels [53]. It is also plausible that gonadotropins, by stimulating ovulation could indirectly promote ovarian cancer development [233].

In contrast, GnRH has been shown to directly inhibit proliferation of normal and malignant OSE cells by increasing the number of cells in the G_0/G_1 phase and promoting apoptosis [246].

Consistent with the gonadotropin hypothesis are:

- the protective effects of OCs and pregnancy, both of which decrease the levels of gonadotropins [53, 233, 245];
- the higher risk with infertility treatment, which is associated with increased gonadotropin levels [238, 242];
- the higher risk in PCOS, a condition associated with elevated LH levels [55, 239, 245, 246]; and
- the observation that the incidence of ovarian cancer peaks 10-20 years after menopause, when gonadotropin levels are elevated [238].

The gonadotropin theory has its shortcomings. Firstly, PMH is associated with higher ovarian cancer risk despite decreasing gonadotropin levels [239, 242, 243, 245]. Secondly, the gonadotropin theory does not explain the inverse association of twin pregnancies with

ovarian cancer, which are usually associated with increased gonadotropin levels [239]. Thirdly, there is an inverse association with breastfeeding although it is associated with elevated levels of FSH [55]. The presence of receptors in ovarian surface epithelial cells for other hormones (progesterone, oestrogen and androgen) suggests that these hormones could also have a role in ovarian cancer development [243].

4.5.2.4 Oestrogens

In OC users, low oestradiol levels, equivalent to early to mid-follicular levels, are maintained; therefore, the oestrogen component of OCs is unlikely to be responsible for the inverse association observed. On the contrary, low levels of oestrogen observed with use of OCs and the associated inverse association supports the concept that oestrogen acts in a facilitative role in ovarian tumourigenesis [53].

Oestrogens have been shown to enhance proliferation of normal and malignant human ovarian surface epithelial (HOSE) cells [238, 249]. Syed et al. reported a dose-dependent, oestrogen-receptor-mediated, growth-promoting effect of oestrogen on normal and malignant HOSE cells. Oestrogen was found to cause an approximately 10-14 fold increase in growth of normal HOSE cells and a 3-4 fold increase in ovarian cancer cell lines. Oestrogen levels comparable to the normal plasma levels in pre-menopausal, as well as in post-menopausal, women were used in this study. Based on these results, normal serum levels of oestrogen in pre-menopausal and post-menopausal women are high enough to promote proliferation of normal and malignant HOSE cells. 17β-oestradiol (E_2) and oestrone (E_1) were also shown to have equal potency in stimulating cell proliferation [238]. This is a surprising, yet important finding. Oestrone, the main oestrogen produced in post-menopausal women is known to be less potent than oestradiol [238, 255]. In line with this, oestrogen formation by adipose-tissue aromatase is thought to play a role in ovarian cancer progression. Higher ovarian cancer mortality rates have been reported in overweight (BMI ≥25) and obese (BMI >30) post-menopausal women [255].

It has also been proposed that oestrogen may promote ovarian carcinogenesis by inhibiting apoptosis of ovarian epithelial cells. This was demonstrated in a study done on macaques (*Macaca fascicularis*), in which those treated with oestradiol alone or Triphasil (Triphasil contains ethinyl oestradiol and levonorgestrel) had lower rates of apoptosis, 1.8% and 14.5% respectively, than the group treated with levonorgestrel alone, 24.9% [248].

It has been argued that it is the hormone levels in the ovarian microenvironment, as opposed to circulating hormone levels, that influence ovarian cancer risk (stromal hypothesis) [255]. Oestrogen production in the follicles rises sharply just before ovulation and, to a lesser extent, at the midluteal phase, after which the levels decrease [53]. Levels of oestrogen in the ovarian stroma are ≥100-fold higher than that in the circulation, with follicular levels being markedly higher still [255]. At the time of ovulation, OSE cells are exposed to follicular fluid that contains concentrations of oestradiol about 10,000 times higher than serum levels [53]. It has been postulated that DNA damage of OSE cells at the ovulation site/CICs may be caused by the exposure to high oestrogen levels [255]. It has also been shown that ovarian cancer cells produce oestrogen, further supporting a paracrine role of this hormone in the development of ovarian cancer [255]. Pregnancy is established as protective against ovarian cancer. During pregnancy, however, the circulating oestrogen levels are about 100-fold higher than the non-pregnant levels, which seems quite inconsistent with a promotional role for oestrogen in ovarian cancer; however, after the first few weeks of pregnancy, hormone production, including oestrogen, is mainly from the placenta [239]. According to the stromal hypothesis, exposure of CICs to high oestrogen levels may lead to neoplastic transformation [245]. Congruent with this, it has been argued that the protection conferred by combined OCs via decreased ovulation can also be attributed to reduced levels of oestrogen within ovarian tissue [255].

Oestrogen is thought to play a promotional role in ovarian carcinogenesis. The observed inverse association with breastfeeding is consistent with this. In breastfeeding, oestradiol and LH levels are decreased, but FSH is elevated. In addition, use of PMH is associated with higher risk of ovarian cancer and the risk may be higher for those who used oestrogen-alone, as opposed to oestrogen-progesterone, therapy [239, 246]. Oestrogen replacement therapy decreases gonadotropin levels but raises circulating oestradiol levels [53].

On the other hand, pregnancy confers protection against ovarian cancer and is associated with high levels of oestrogen, increased about 100-fold, and high hCG levels (which may exert a gonadotrophic effect), suggesting that another hormone may be involved in ovarian carcinogenesis [53]. Furthermore, inhibition of ovulation and reduced gonadotropin and oestrogen levels may not be the only mechanisms responsible for the protective effect of OCs, which contain progesterone and have also been shown to affect levels of androgens.

4.5.2.5 Progesterone

Oral contraceptive use results in a reduction in endogenous progesterone production to levels similar to those present during the early follicular phase [53]. However, OCs contain high levels of synthetic progestins, with potencies considerably higher than that of progesterone. Plasma levels of progestins in OC users are equivalent to luteal-phase levels of progesterone [55]. Progesterone has been shown to have a protective role in ovarian carcinogenesis and it has been suggested that the progestin component of OCs importantly contributes to lower risk of ovarian cancer [53, 248].

The protective effect of progesterone is thought to be mediated via decreased proliferation and increased apoptosis [255]. Progesterone has been shown to inhibit proliferation of cultured OSE cells from both pre- and post-menopausal women [55]. In a study in which different levels of progesterone were used, progesterone at low doses, equivalent to normal pre-menopausal plasma levels, enhanced proliferation of normal and malignant epithelial cells; however, at higher doses, equivalent to levels observed during pregnancy or mid-luteal phase, it had a growth-inhibitory effect [238]. The effect was dose dependent and mediated via progesterone receptors. This is in line with the observation that repeated ovulatory cycles are associated with increased ovarian cancer risk, whereas pregnancy is protective [238].

Progesterone induces apoptosis in normal and malignant human ovarian surface epithelial cells [246]. In two human ovarian cancer cell lines (3AO and AO), examined 3 days after exposure to progesterone at 10μM, the proportion of cells in G_1 phase had increased from 37.0% to 72.5% and 53.5% to 73% respectively. This was accompanied by a drop in the proportion of cells in S-phase from 31.9% to 11.3% and 26.6% to 13.4% respectively. In addition, the proportion of progesterone-treated cells undergoing apoptosis was more than 50% compared to less than 3% in controls. The effect was dose dependent. Concurrently, there was an increase in the level of *p53* mRNA with no effect on *bcl-2* or *c-myc*, suggesting that *p53* may be responsible for these effects. *p53*, a tumour suppressor gene, among other multiple roles, induces apoptosis and cell-cycle arrest [256].

In a 3-year experimental study on macaques (*Macaca fascicularis*), using Triphasil, a combined oral contraceptive containing levonorgestrel and ethinyl oestradiol, progesterone was shown to cause apoptosis. In this study, the primates were divided into 4 treatment groups: Triphasil, levonorgestrel only, ethinyl oestradiol only, and no hormones (control group). A statistically significant increase in apoptosis of ovarian epithelial cells was

observed in the Triphasil ($P \leq 0.01$) and progesterone groups ($P < 0.001$) versus the controls and oestradiol groups, up to four-fold and six-fold higher respectively. This was despite the fact that a majority of the monkeys in the control group were in luteal phase (70%, 14 out of 20) [248]. The increased apoptosis in ovarian epithelial cells was accompanied by a switch in the expression of TGF-β isoforms from TGF-β_1 to TGF-β_2/β_3. Treatment with progestin resulted in a statistically significant decrease in TGFβ_1 expression ($P < 0.001$) and an increase in TGFβ_2/β_3 expression ($P < 0.001$) in ovarian epithelial cells. These findings suggest that TGFβ plays a role in the apoptotic process. [243] Ovarian surfaces of progestin-treated monkeys also showed patches without an epithelial lining [248].

By increasing apoptosis of ovarian epithelial cells, progestins may increase the likelihood that cells that have sustained DNA damage will be eliminated, thus decreasing the risk of ovarian cancer [248].

The following observations are compatible with the proposition that progesterone is protective. First, the presence of progesterone receptors on ovarian epithelial cells and ovarian cancer cells suggests that this hormone may have a role in ovarian tumour biology [53]. ER and PR have been shown to be expressed by both normal OSE and ovarian cancer cells, with a reduction in PR and ERα in ovarian cancer cell lines compared to OSE cells [255]. Second, the risk of ovarian cancer is inversely related to parity [246]. The inverse association with pregnancy surpasses that which can be attributed to ovulation inhibition and has been attributed to the high levels of progesterone in pregnancy [53]. Third, compared to singleton pregnancies, the reduction in risk of ovarian cancer may be higher in women with dizygotic twins [53, 239, 255], with one study reporting a total parity-adjusted OR of 0.68 (95% CI = 0.33-1.38) [53]. This is despite these women having higher gonadotrophin levels and being more likely to double-ovulate during their reproductive years than women with singleton pregnancies only. This protection has been attributed to higher progesterone levels in twin pregnancies [53, 239, 255]. The high levels of progesterone are thought to cause exfoliation of cells thereby reducing the ovarian burden of cells with genetic damage [238]. Consistent with this, studies have reported lower risk of ovarian cancer with older age at first and last birth [239]. Furthermore, use of PMH is associated with higher risk of ovarian cancer and the risk may be higher for those who used oestrogen alone as opposed to oestrogen/progesterone combinations [239, 246].

The other hormones thought to play a role in ovarian carcinogenesis are androgens. Androgen receptors are expressed by most ovarian cancers (90%), whereas oestrogen and progesterone receptors are expressed by 55% and 52% of ovarian cancers respectively [257].

4.5.2.6 Androgens

High androgen levels are thought to play a facilitating role in ovarian cancer development [53, 245]. High levels of androgens are produced by developing follicles [235]. Androgen is the hormone with the highest concentration in the follicular fluid, being 10 times higher than that of oestradiol in early follicles (<10mm) [53]. Therefore, CICs are exposed to an androgen-rich microenvironment during the follicular phase [53, 235]. Furthermore, follicles that later undergo atresia continue to produce androgens. Around the time of ovulation, there is increased production of oestrogen by the dominant follicle. [53] Use of OCs results in a decrease in ovarian testosterone production and this is thought to contribute to its protective effect [53, 55, 239]. These observations lend further support to the stromal hypothesis [239]. Oestrogens present in OCs also cause an increase in SHBG, thus decreasing free testosterone levels. SHBG has a greater effect on free testosterone levels than on oestradiol [237].

Androgens have been shown to promote proliferation of normal OSE cells [239]. In a study done by Syed et al, both dihydrotestosterone (DHT) and testosterone (T) enhanced cell proliferation in ovarian cancer and HOSE cells. However, DHT was more effective in enhancing cell growth of normal OSE than T. Similar effects were observed in ovarian cancer cell lines. The effect was dose dependent and mediated via hormone-specific receptors [238]. In a nested case-control study, cases were found to have higher androstenedione levels and DHEA levels, but to show no difference in DHEA sulphate levels compared with controls. Androstenedione is converted in the ovaries to the more potent DHT, T, and oestrogen. However, no difference in androgen levels was observed among postmenopausal women [239].

In support of the role of androgens increasing ovarian cancer risk, there is a higher risk of ovarian cancer in women with PCOS, hirsutism, and acne, conditions associated with elevated androgen levels [53, 55, 235]. In PCOS, the LH and androgen levels are raised, whereas FSH levels are low [45]. The increased risk of ovarian cancer in post-menopausal or obese women has also been linked to increased androgen levels [245]. During menopause, the raised gonadotropin levels stimulate production of androgens from the ovary, resulting in an androgen-rich microenvironment [238, 239].

4.5.3 LONG-ACTING PROGESTOGEN-BASED CONTRACEPTIVES

Long-acting progestogen-based contraceptives exert effects similar to those suggested to be responsible for the protective effect of OCs. Most importantly, these contraceptives inhibit ovulation. Use of DMPA also leads to a decrease in plasma oestrogen to levels similar to that found in the early to mid-follicular period (100pg/mL) [53]. Lower levels oestrogen, similar to post-menopausal levels (100pmol), have been reported in users experiencing amenorrhoea [12]. Although DMPA inhibits endogenous progesterone production [53], use of DMPA results in high levels of plasma progesterone (1ng/mL) for the 3 months following injection [12, 53]. DMPA inhibits the mid-cycle LH surge but, overall, does not affect gonadotropin levels [53]. From the above, the overall effect should be a reduced risk of ovarian cancer if, indeed, OCs confer protection through these proposed biologic mechanisms.

On the other hand, it has been suggested that DMPA may not have the same effect as the progestin components of oral contraceptives because they do not belong to the same class of progestins. Most of the progestins in combined OCs are 19-nortestosterone derivatives, whereas DMPA is a progesterone derivative [248].

It has also been suggested that DMPA may have an androgenic effect, which may therefore, increase the risk of ovarian cancer. In an experimental study, medroxyprogesterone acetate (MPA) at pharmacologic doses (equivalent to that used in the treatment of breast and endometrial cancer) was found to be more effective in promoting the invasiveness of EOC cells than DHT. In this study, the EOC cell line had AR receptors and no PR receptors, and assessment was done after 24 hours of exposure; this limits generalisation on the wider effects of MPA on disease progression, and the effect of long-term use of progestins in women [257].

4.5.4 INTRAUTERINE CONTRACEPTIVE DEVICES (IUDS)

Use of IUDs is thought to increase the risk of infection and inflammation in the peritoneal cavity; this, in turn, leads to a higher risk of ovarian cancer [7]. Chronic inflammation, including subclinical inflammation, has been shown to increase the risk of cancer in affected organs [234, 258, 259]. It has been argued that it is the ensuing inflammatory response, rather than the cause of the inflammation, that predisposes to cancer [258]. Leukocytes recruited during the inflammatory process release reactive nitrogen and oxygen species, which can cause genetic mutations [258]. Prolonged inflammation allows enough time for the acquisition of an adequate number of mutations needed to drive the carcinogenesis process [234]. Cells involved in the inflammatory process also produce substances that inhibit

apoptosis, promote cell proliferation and angiogenesis, and cause perturbation of host immune responses. All these factors increase the likelihood of cancer development [258]. Furthermore, due to local stress, there is continuous cell death (mainly necrosis due to impaired apoptosis) at inflammatory sites. This results in repair processes, including cell proliferation and angiogenesis, which promote carcinogenesis [234]. In addition, the cellular contents released during cell necrosis further promote inflammation [234].

Consistent with the inflammatory hypothesis in ovarian carcinogenesis is the observed lower risk with prolonged use of low-dose aspirin, and other non-steroidal anti-inflammatory agents [55, 235]. Exposure to inflammatory factors such as asbestos and talc has been linked to ovarian cancer although available evidence is inconclusive [55, 246]. The inflammatory hypothesis is also supported by the higher risk of ovarian cancer in women with PID and endometriosis [245].

Pending further evidence and considering the hypotheses of ovarian carcinogenesis, it is reasonable to hypothesize that Cu-IUDs, which cause an inflammatory reaction, increase the risk of ovarian cancer, whereas LNG-IUS have the same consequences as other long-acting progestogen-based contraceptives, namely a lower risk.

4.5.5 VASECTOMY

4.5.5.1 Normal semen composition

Semen is formed by the confluence of secretions from many glands including the testis, prostate, seminal vesicles, Cowper's glands, and periurethral glands [260-262]. There is also contribution from the epithelia of the male genital tract [260]. The normal volume of semen per ejaculate is 3 ml (2-6 ml) [262]. Semen consists of a cellular portion (represents 2-5% of total volume), which is mainly composed of spermatozoa (others are white blood cells and epithelial cells), and seminal plasma (acellular portion). The prostate contributes 15-30% of the total seminal volume, while 50-80% is contributed by the seminal vesicles [260, 262]. Seminal plasma contains fructose, prostaglandins (mainly PGE and PGF), zinc, citric acid, carnitine, glycerophosphocholine, lipids, minerals, enzymes, and proteins [260, 262]. The secretory functions of the male accessory reproductive glands (ARGs) are influenced by testosterone [260, 262]. Not all substances secreted into the semen, especially by the more proximal part of the tract (vas, testis, and epididymis) are eventually present in ejaculated semen; this is due to reabsorption by the genital tract epithelia and at times incomplete voiding [260].

Prostaglandins and fructose are mainly produced by the seminal vesicles, and therefore, the seminal plasma content of these substances can be used to assess the function of the seminal vesicles, although fructose is also produced by the ampullary glands [260]. Further, citric acid, zinc, and acid phosphatase are produced by the prostate and can therefore, be used to assess its function [260]. The prostate also produces polyamines (spermine, spermidine, and putrescine), which promote growth, and have antimicrobial effects [260]. Semen has the highest concentration of prostaglandins in the human body fluids [260]. Prostaglandins in semen stimulate muscle contractions, thus aiding the passage of semen through the female genital tract. Other substances that also cause muscle contractions and therefore aid in seminal passage, are oxytocin and noradrenaline [260]. Motility of spermatozoa and unidirectional movement are also influenced by mid-cycle cervical mucus, which has linear longitudinal channels that facilitate upward movement [260].

The testes produce androgens and oestrogens. Testosterone is converted to DHT in a process catalysed by 5-α-reductase. The testes also produce androgen-binding protein (ABP) which acts as a carrier for testosterone in semen. Concentration of testosterone in seminal plasma is lower than, or equal to, that in serum, whereas the levels of oestrogens are higher in semen than those in blood. DHT levels are higher than testosterone levels in semen. Testosterone levels have no relationship with sperm density; however, DHT levels are low in oligospermia and azoospermia, and are no longer present after vasectomy. Other hormones found in human seminal plasma are FSH, LH, and anti-Müllerian hormone (AMH). The levels of FSH and LH in seminal plasma are similar to those in blood plasma, and do not vary according to sperm density [260].

The membrane of the spermatozoon binds some of the constituents of semen including minerals (such as calcium and zinc) and hormones [260]. In light of the contribution from many organs, each with distinct biochemistry, semen composition is affected by change in the relative contribution of the accessory reproductive glands [260, 262].

4.5.5.2 Effect of semen on the female

Little is known about what happens to semen after deposition into the female genital tract. However, seminal fluid contains hormones, prostaglandins, glycoproteins (including cytokines and growth factors), which can trigger both local and distant responses in the female [263]. In insects, secretions of the male ARGs have been shown to affect all aspects of reproductive function in the female, from ovulation to oviposition and post-mating

refractoriness [264]. The same may occur in mammals. Absorption of semen constituents occurs soon after deposition in the female genital tract. Effects on a woman are thought to include impacts on central nervous system (CNS) and behaviour [263].

Deposition of semen into the female genital tract leads to an inflammatory reaction in the cervix and uterus. No response is observed with use of condoms. This reaction may also occur in the upper genital tract and ovaries because spermatozoa usually bind constituents of seminal fluid and these may therefore facilitate their access to the upper genital tract. In addition, hormones and inflammatory factors may be transported through blood vessels from the cervix to the endometrium [263]. Factors found in human semen that may induce inflammation include PGE, IL-8, and bacteria (semen has an abundance of bacteria of different species) [263]. Secretions from the seminal vesicles are important in this as demonstrated by the presence of an immune response in vasectomised males and its absence after removal of seminal vesicles [263]. This immune reaction is important in:

- eliminating micro-organisms and excess spermatozoa (maintenance of sterility of the upper genital tract is important for survival of the spermatozoa);
- stimulation of antigen-specific (paternal-specific) immune tolerance, which is necessary for embryo survival;
- endometrial remodelling in readiness for implantation; and
- the implantation process [263].

Seminal plasma antigens include blood-plasma proteins (albumin and globulin) and semen-specific antigens [260]. Evidence of sperm antigenicity includes:

- production of sperm autoantibodies which sometimes leads to infertility [260];
- tolerance of the foreign conceptus (exposure to sperm allows women to develop tolerance to the paternal elements of the conceptus) [263]; and
- the fact that the immune response is also involved in the development of a hydatidiform mole and preeclampsia [23].

In addition, hormones (which include oestrogen, androgens, FSH, and LH) that are present in semen are bound to receptors in the female genital tract [265].

4.5.5.3 Changes in semen composition post-vasectomy

Following vasectomy, contributions from testes, epididymis, and part of the vas to the seminal plasma are no longer present in semen [260]. There is also a reduction in the

substances produced by the prostate [266, 267] and a decrease in semen volume [266, 268]. In contrast, the function of seminal vesicles, which are the principal source of prostaglandins, does not seem to change post-vasectomy [266, 267, 269]. In a study in which semen of 56 men who were 8 years post-vasectomy and an equal number of non-vasectomised men of similar age who had fathered at least one child were compared, a decrease in volume of semen (3.0 versus 4.9 ml; P <0.01) and secretory products of prostatic origin (zinc, magnesium, prostatic acid phosphatase [PAP], and citric acid), but not seminal vesicular origin (PGE1 and fructose) was observed. There was also a statistically significant lower concentration of spermidine (366 versus 650 nmol; P <0.005) and spermine (5,435 vs 11,804 nmol; P <0.05), but not putrescine in the vasectomised men. Polyamines (putrescine, spermidine, and spermine) are mainly produced by the prostate gland [266]. Similarly, the maltase activity in semen of 35 men who underwent vasectomy 1-2 years before the study was significantly lower relative to 25 men with intact vas deferens of the same age and socio-economic status (11.7 versus 28.7; P <0.001) [267]. Maltase is produced by the prostate under the influence of testosterone. No difference in fructose levels, which was used as a measure of function of the seminal vesicles, was observed between vasectomised and non-vasectomised men [267]. No relationship between the level of these substances and duration of vasectomy was reported [268].

It is not yet clear whether vasectomy affects the function of the pituitary-gonadal axis, although blood plasma levels of FSH, LH, and testosterone have been reported to be the same before and after vasectomy [265, 267]. However, there may be changes in the levels of hormones in seminal plasma [270]. In a longitudinal study of 19 healthy men in which measurement of serum hormone levels and assessment of the function of accessory organs was done on the day of vasectomy and 3 months and 1 year after vasectomy (participants were aged 35-45 years) [265], no changes in blood serum FSH, LH, and testosterone levels following vasectomy were observed. However, there was an increase in seminal fructose and a decrease in maltase activity and citric acid [265]. Similarly, in a study in which 19 healthy Danish men (30 to 62 years of age), of proven fertility had blood and seminal plasma steroid hormones levels analysis prior to vasectomy and at 4 monthly intervals for 2 years post-vasectomy, no changes were observed in blood plasma concentrations of the steroid hormones [270]. A statistically significant decrease post-vasectomy in the seminal plasma levels of DHT (40%) and T (23%) was observed as well as a slight decrease in the levels of DHT-S (9%) and DHEAS (8%), whereas no change was observed in the levels of other

steroid hormones [270]. There was no relationship between time since operation and levels of steroids; however, there seemed to be an initial decrease in the levels of E_2 followed by a return to normal [270]. The lack of alteration in the levels of other steroid hormones suggests that they are produced by the accessory glands (seminal or prostatic source), whereas the decrease in DHT and T post-vasectomy suggests that they are mainly produced by the testis and/or epididymis [270].

Reduction in the prostatic contribution post-vasectomy may be due to decreased levels of testosterone reaching the prostate from the testes due to a break in vascular continuity [266, 268]. Maltase is produced by the epididymis and, therefore, a reduction post-vasectomy is expected [266]. On the other hand, vasectomy does not seem to affect the secretory function of the seminal vesicles as reflected in no changes in levels of PGE and fructose, which are predominantly produced by the seminal vesicles [266].

4.5.5.4 Biologic mechanisms of the association between spousal vasectomy and the risk of ovarian cancer

How and why spousal vasectomy may modify the risk of ovarian cancer have not been determined. From the above discussion and knowledge of the pathogenesis of ovarian cancer, the following biologic mechanisms are proposed.

The constituents of semen, which have the potential to cause inflammation, partly gain access to the upper genital tract via facilitation by spermatozoa. Spermatozoa have the ability to bind most of the seminal constituents [260]. Infectious agents from the lower genital tract are thought to be responsible for upper genital tract infection [45]. Spermatozoa have been proposed as one of the factors that facilitate the ascent of bacteria from the lower to the upper genital tract [222]; in their absence, a lower risk of infection and inflammation could be predicted and, thus, lower risk of ovarian cancer.

Semen from a fertile man contains 20 million spermatozoa per mL or more [23]. Following sperm deposition into the female genital tract, other than the sperm that fertilizes the ovum, the rest undergo degeneration [22]. Cell necrosis leads to the release of cellular contents which may cause inflammation [58, 234]. As previously discussed, inflammation causes an increased risk of cancer. Therefore, if the spermatozoa present in the female genital tract undergo necrosis, this may increase the risk of cancer.

Vasectomy results in the reduction of seminal plasma content of DHT and T. In addition, the absence of spermatozoa, which usually bind hormones in semen including testosterone, FSH,

LH, and oestrogen could lower risk because of the lower levels of these hormones in the upper genital tract.

A reduction in seminal constituents of prostatic origin following vasectomy has been demonstrated. These include the polyamines (spermine and spermidine). Oxidation products of polyamines may have a similar effect on risk of ovarian cancer as they do in cervical cancer. Although HPV infection is linked to the development of cervical cancer, not all women with HPV develop cervical cancer; there are also other risk factors including smoking, use of OCs, parity, and HIV co-infection [23]. It has been suggested that the presence of diamine oxidase (DAO) and polyamine oxidase (PAO) in cervical mucus, and spermine, spermidine, and putrescine in the semen of male partners may contribute to the progression from HPV infection to cervical cancer [271]. This is supported by the observation that having long-term (≥3-months) sexual partners as opposed to short-term partners contributes to an increased risk of cervical cancer. The polyamines spermine and spermidine, and the diamine, putrescine are oxidised by PAO and DAO in the female genital tract (cervical mucus). Their oxidative products include oxygen radicals and hydrogen peroxide, acrolein, and reactive aldehydes which are genotoxic and may also have immunosuppressive effects. However, in one study, not all women assessed had DAO/PAO in their cervical mucus (more than half of 126 women assessed had either or both). This may be due to genetic differences or menstrual-cycle phase [271].

When assessing the association between vasectomy and the risk of ovarian cancer, one should guard against selection bias. This is because vasectomy - a permanent contraceptive method - is usually done at an older age after the completion of childbearing, therefore, higher parity and use of OCs may be more prevalent in women whose partners undergo vasectomy.

4.6 USE OF CONTRACEPTIVES AND THE RISK OF CANCER

4.6.1 INTRODUCTION

Two of the central questions when choosing or discontinuing use of a contraceptive method are negative consequences and beneficial effects. Beyond the obvious question of the efficacy of any contraceptive method, it can be argued that the most important of these is possible impact on the risk of cancer, which is both a personal and a public-health issue. We have already discussed the impact of use of contraceptives on the risk of ovarian cancer; however, from both these perspectives, the net-effect on cancer incidence is of greater significance. The association between oral contraceptives and the risk of cancer has been studied most.

Other methods of contraception may also influence risk of cancer. A brief discussion on the association of use of contraception and the risk of cancer, other than ovarian, follows.

4.6.2 USE OF COMBINED ORAL CONTRACEPTIVES AND THE RISK OF CANCER

4.6.2.1 Breast Cancer

Use of hormonal contraceptives may influence the risk of breast cancer, this being a hormonally sensitive tumour. In a 1996 collaborative reanalysis of 54 studies in 24 countries, use of combined OCs was associated with higher risk of localised breast cancer in current and recent users (current users: RR = 1.24; 95% CI = 1.15-1.33, 1-4 years and 5-9 years after cessation of use RR = 1.16; 95% CI = 1.08-1.23, and RR = 1.07; 95% CI = 1.02-1.13, respectively). No association was observed 10 years after cessation of use, (RR = 1.01; 95% CI = 0.96-1.05). In addition, there was no difference in RR of breast cancer in relation to type of combined OCs formulation (oestrogen and progestagen type), dosage, duration of use, or family history of breast cancer. Furthermore, compared to never-users, breast cancer diagnosed in ever-users of combined OCs, including those diagnosed 10 or more years after discontinuation of use, were more likely to be localized (RR = 0.88; 95% CI = 0.81-0.95); hence, there was a better prognosis for ever-users compared to never-users of combined OCs. In this study, there were limited data regarding use beyond 20 years and participants who had stopped use ≥10 years ago were more likely to have used medium- or high-dose preparations [272]. In a subsequent review article, most of the studies reported no increase in the risk of breast cancer among oral-contraceptive users. The few that reported an elevated risk, showed a progressive decline in the strength of this association following discontinuation of use. In addition, risk did not differ according to the composition of OCs used [231].

Consistent with the above findings, a case-control study in South Africa reported a higher risk of breast cancer in recent users (within 10 years) of injectable or oral contraceptives (OR = 1.66; 95% CI = 1.28-2.16, P <0.001), with no difference in risk 10 or more years after last use (OR = 1.11; 95% CI = 0.91-1.36, P = 0.3) compared to never-users. There was also no relationship between risk and duration of use of hormonal contraceptives (P = 0.4). A trend toward lower risk with longer duration since last use was observed (P = 0.004). Of note is that, in this study, oral contraceptives were assumed to be combined oral contraceptives, whereas injectable contraceptives were assumed to be progestogen-only preparations. However, there was no statistically significant difference in risk among recent users of injectable contraceptives exclusively (OR = 1.83; 95% CI = 1.31-2.55), oral contraceptives

exclusively (OR = 1.57; 95% CI = 1.03-2.40), and users of both (OR = 1.50, 95% CI = 1.04-2.17), P - heterogeneity = 0.6 [12].

In contrast, a cohort study found no increase in risk of breast cancer among users of oral contraceptives; on the contrary, an inverse association was observed with onset of use at less than 29 years of age (HR = 0.68; 95% CI = 0.46-1.00), compared to never-use. Participants in this study had used oral contraceptives for a short time, the median duration of use being 2 years. In addition, the study had low power and lacked information on specific type of OCs used [241].

The association between OCs and breast cancer could be attributed to detection bias (increased surveillance). Because women using OCs visit health facilities to renew their prescriptions, they are more likely to undergo a physical examination and therefore benefit from possible early detection of breast cancer [236]. On the other hand, OCs may simply affect the rate of growth of tumours that are already present. Indeed, the observation that higher risk is confined to the first 10 years after discontinuation of use and the lack of association of risk with duration of use of combined OCs are both more in support of a promotional effect than a genotoxic effect [272]. Oestrogens are thought to promote proliferation of ductal epithelial cells leading to an increase in the risk of DNA errors or replication of cells with genomic damage; progestagens may have a synergistic effect [12].

In conclusion, studies suggest that the increase in risk of breast cancer among OC users is minimal or absent and is confined to current or recent users [231]. The absolute risk of breast cancer in ever-users compared with never-users is small. For instance, for the period from onset of use until 10 years after cessation of use, there will be an extra 5 breast cancer cases (49 instead of 44) per 10,000 women if combined OCs are used for 5 years by women at the age of 25 years [272]. Overall, the proportion of breast cancer cases that can be attributed to the use of OCs is less than 1%; however, for pre-menopausal women the association is stronger (about 7%) [231]. Regarding mortality, Vessey et al. observed no relationship between breast cancer mortality and ever-use or duration of use of combined OCs (RR = 1.0; 95% CI = 0.8 - 1.2, for ever-use) [273].

4.6.2.2 Cervical Cancer

Long-term use of combined OCs (≥5years) by women with HPV infection may result in a higher risk of cervical cancer. A pooled analysis reported a statistically significant higher risk of cervical cancer in ever-users of OCs (OR = 1.47; 95% CI = 1.02-2.12) with a trend toward

higher risk with longer duration of use (use for <5 years: OR = 0.77; 95% CI = 0.46-1.29, 5-9 years: OR = 2.72; 95% CI = 1.36–5.6, and ≥10 years: OR = 4.48; 95% CI = 2.24-9.36), although the p-value for trend was not reported. The risk returned to normal 5 to 10 years after discontinuation of use [274]. These findings are corroborated by those of Cibula et al.: use of OCs for more than 5 years was associated with a higher risk of cervical cancer. The strength of this association declined with longer duration since cessation, and was almost absent after 10 years [231].

The findings of Urban et al. are consistent with this: they reported a statistically significantly higher risk of cervical cancer in recent users (within 10 years) compared to never-users of hormonal contraceptives (OR = 1.38; 95% CI = 1.08-1.77; P = 0.01). A clear decline in risk with longer time since last use was observed (P = 0.02), with a return to background risk 10 or more years after discontinuation of use (OR = 1.01; 95% CI = 0.84-1.22). However, no association was observed with duration of use (P = 0.96) [12].

A similar relationship between OC use and mortality was observed in ever- versus never-users (OR = 7.3; CI=1.2-30.5) with a statistically significant trend toward higher risk with longer duration of use (P = 0.004) [273].

4.6.2.3 Endometrial Cancer

It is well recognised that combined OCs are inversely associated with risk of endometrial cancer [231, 240, 275]. Oral contraceptives have to be used for at least 4 years for this beneficial association to emerge. Duration of use is inversely related to risk with a 50% lower risk after 4 years of use and about 70% after 12 years of use [240]. In one study, no inverse association was observed with use for <5 years (OR = 1.28; 95% = 0.71-2.32; P = 0.4), whereas a statistically significant lower risk (OR = 0.44; 95% CI = 0.22-0.86; P = 0.002) was seen with use for ≥5 years (P-heterogeneity = 0.007 for duration of use) [12]. The trend is toward progressively higher risk after cessation of use; however, the risk of endometrial cancer is still lower for ever-users than never-users long after cessation, with up to a 50% lower risk 20 years after discontinuation [231, 240]. The inverse association with endometrial cancer is most probably due to suppression of endometrial proliferation by progestogen [12, 231, 275].

In a prospective cohort study, ever-users of OCs had a statistically significantly lower risk of mortality from uterine cancer, which were mostly endometrial, (RR = 0.3; 95% CI = 0.1-0.8). A progressively lower risk was observed with longer duration of use (P-trend = 0.002): those

who had used OCs for more than 8 years had a RR of 0.2 (95% CI = 0.0-1.0). This beneficial association was still present 20 years after cessation of OC use (RR = 0.4, 95% CI = 0.1-1.0) [273].

4.6.2.4 Other Cancers

Ever-use of combined oral contraceptives is associated with a 20% to 30% lower risk of colorectal cancer [276]. OCs may be associated with a higher risk of hepatocellular adenoma; however, this condition is rare (prevalence of 3 to 4/100,000), and risk seems to be associated with duration of use and oestrogen levels in the contraceptive preparation [224]. The risk of hepatocellular carcinoma, also a rare condition, is slightly higher in oral contraceptive users: in a pooled analysis, the relative risk of hepatocellular carcinoma was 1.70 (95% CI = 1.12-2.59); the risk seemed to be higher with longer duration of use and returned to normal after discontinuing OC use [231].

A prospective cohort study reported a statistically significant positive association between ever-use of oral contraceptives and gallbladder cancer (HR = 2.38; 95% CI = 1.26-4.49), but there was no association with rectal, colon, or gastric cancer. These findings were based on a small number of site-specific cancers [241]. Not enough evidence is available to adequately assess the impact of use of OCs on the risk of other cancers [224, 231]. Overall, mortality from all cancers is lower in ever- compared to never-users of OCs (RR = 0.87; 95% CI = 0.79-0.96) [273].

4.6.3 USE OF DMPA AND THE RISK OF CANCER

4.6.3.1 Breast Cancer

The relationship between DMPA use and the risk of breast cancer is thought to be similar to that of OC use [277]. In a New Zealand case-control study, overall, DMPA was found to have no association with risk of breast cancer (RR = 1.0; 95% CI = 0.8-1.3) and no relationship between risk and time since first use or duration of use. However, a higher risk was observed in DMPA users younger than 35 years of age and in recent users. A linear relationship between duration of use and risk was also noted in this group of young women. Generally, a lower risk was observed with longer duration since last use. These findings suggest that use of DMPA by young women may be associated with a higher risk of breast cancer. The observed higher risk in the first few years of use supports a promotional effect on tumours that are already initiated [278].

In a pooled analysis, which included the above study, the findings were generally comparable to those for OC use. A higher risk of breast cancer was observed in recent users (within 5 years) of DMPA (RR = 1.17; P = 0.06, for oral contraceptives, and RR = 1.17; statistically non-significant, for injectable contraceptives). There was also no association with duration of use or age at first use. A small number of participants had used progestagen-only preparations in this study (0.8% had used OCs and 1.5% had used injectable preparations) [272].

Urban et al. also observed no statistically significant difference in risk of breast cancer between users of injectable or oral contraceptives. The risk was higher in ever-users of hormonal contraceptives than never-users; no relationship with duration of use was observed; and risk trended lower with time since last use [12].

4.6.3.2 Cervical Cancer

Overall, use of DMPA does not seem to be associated with the risk of cervical cancer [277, 279]. However, there may be a higher risk in recent users [12]. In a review article, no association was found between DMPA use and the risk of cervical cancer. Two case-control studies reported relative risks of 1.4 (95% CI = 0.6-3.1) and 1.1 (95% CI = 0.96-1.29). There was also no association between risk and length of use or time since cessation of use [277]. A case-control study by Urban et al. reported a statistically significantly elevated risk in women who had recently used (previous 10 years) injectable contraceptives exclusively compared to those who had never used hormonal contraceptives (OR = 1.58; 95% CI = 1.16-2.15, P = 0.004). No statistically significant difference in risk was observed in recent users of injectable contraceptives only, oral contraceptives only, or both (P-heterogeneity = 0.2) [12]. These studies did not include data on HPV infection which is a necessary cause of cervical cancer.

4.6.4 USE OF OTHER CONTRACEPTIVES AND THE RISK OF CANCER

Use of barrier methods decreases the risk of cervical cancer by reducing HPV transmission [23, 231, 277]. Use of IUDs has no impact on the risk of cervical cancer [231]. In one study, IUD use was associated with a lower risk of breast, thyroid, lung, ovarian, and uterine body cancer, and a higher risk of rectal and stomach cancer, whereas tubal ligation was associated with a higher risk of uterine body cancer and was inversely associated with stomach cancer. Although tubal ligation is known to be inversely associated with ovarian cancer risk, the study did not demonstrate any association between the two. The findings of this study could be attributed to chance [241].

4.6.5 NET EFFECT OF USE OF CONTRACEPTIVES ON THE RISK OF CANCER

If extending the indications of use of contraceptives to include prevention of ovarian cancer were to be considered, a deterrent would be a deleterious effect on the overall risk of cancer and other morbidities. It is therefore encouraging that, so far, use of contraceptives has not been associated with a higher risk of cancer; if anything the net association is inverse. Studies have reported a decrease in the incidence of cancer by 10 to 45 per 100,000 women per year in OC users [224]. In a cohort study, ever-use of any method of contraception was not associated with a higher overall risk of cancer (HR =1.02; 95% CI = 0.92–1.12) [241]. Similarly, the Oxford-Family Planning Association (Oxford-FPA) contraceptive study observed a lower risk of mortality from cancer in ever-users of oral contraceptives (RR = 0.9, 95% CI = 0.8-1.0), the relative risk for all-cause mortality was also reduced (RR = 0.87; 95% CI = 0.79-0.96) [273]. The RCGP Oral Contraception Study reported an absolute reduction in mortality of 52 per 100,000 woman years among women who had ever used OCs. The overall death rate was also statistically significantly lower (RR = 0.88; 95% CI = 0.82-0.93) [280]. The benefits from preventing unwanted pregnancies, especially in developing countries, cannot be over-emphasised.

4.7 CONCLUSION

Cancer of the ovary is mostly detected at an advanced stage, with poor treatment outcomes [230]. Prevention is critical in combating this disease. Oral contraceptives have been shown to decrease ovarian cancer risk. Demonstration of other mechanisms of action underlying the protective effect of oral contraceptives, other than inhibition of ovulation, may lead to the development of a preventive agent that is more effective than OCs, and can also be used by post-menopausal women [243].

Current evidence suggests a promoting role for high levels of androgens, oestrogens, and gonadotropins, whereas ovulation inhibition and high progesterone levels are inversely associated. However, it is difficult to tease apart the effects of the different hormones on risk of ovarian cancer because they are closely interrelated [239]. These hormones have a common synthetic pathway. In addition, circulating levels of one hormone may influence the production of another, by exerting negative or positive feedback on the hypothalamo-pituitary-ovarian axis. For example, androgens are a precursor of oestrogens, high levels of oestrogen result in inhibition of gonadotropin production, whereas high gonadotropin levels drive the production of oestrogens and androgens. In addition, ovulation, which is thought to play an important role in ovarian cancer causation, affects the levels of various hormones. In

pre-menopausal women, anovulation results in high levels of androgens and oestrogens and low levels of progesterone. Furthermore, established risk factors support more than one hypothesis of ovarian cancer pathogenesis. Finally, steroid-hormone receptors, although they preferentially bind to specific ligands are, nonetheless, somewhat promiscuous and can bind and transmit signals from more than one hormone.

In view of current evidence supporting the protective role of progesterone, this hormone could be used in prevention. It has been proposed that high levels of progesterone, equivalent to those found in pregnancy, could be administered periodically to women at high risk of ovarian cancer so as to trigger apoptosis of OSE and therefore, promote loss of cells that may have undergone genetic damage [255]. If used at all, it may also be safer to use progesterone in combination with oestrogen as PMH [238]. Progesterone could also be used in combination with other cytotoxics in the treatment of ovarian cancer [255].

Tamoxifen (a second generation anti-oestrogen) has been used as a second-line treatment in patients with ovarian cancer with little success (5-18% response rates) [230, 242, 255]. These poor results could be attributed to choice of patients in clinical studies; tamoxifen is used as a treatment of last resort [230, 242]. As response to treatment is related to the level of ER, another factor could be low levels of ER receptors in ovarian cancer cells [242, 255]. It is also not clear whether tamoxifen acts as a pure antagonist of oestrogen on ovarian tissue or if it also has an agonist effect [230]. Tamoxifen used in combination with platinum-based chemotherapy has shown a better response (overall response of 50%) [255]. GnRH analogues, progestins, anti-androgens, and letrozole have also been used in treatment of recurrent ovarian cancer with poor results [246]. Letrozole, an aromatase inhibitor, has been shown to increase the levels of ER in ovarian tumours which may promote the response to anti-oestrogens [255]. It has also been proposed that oestrogen-regulated gene products including fibulin-1 and cathepsin D, and ERβ-negative status could be used as early biomarkers of ovarian cancer [230].

In summary, oral contraceptives and tubal ligation are inversely associated with, and possibly protective against, ovarian cancer, whereas the impact of other contraceptive methods on ovarian cancer risk has not yet been established. From the above discussion, it is conceivable that long-acting progestogen-based contraceptives and spousal vasectomy would be protective, whereas Cu-IUDs would increase risk. These are the primary hypotheses driving the current research, on the basis of which, we carried out a study to assess the impact of use

of various contraceptives on ovarian cancer risk. In the subsequent chapters, the methods and results of a population-based case-control study assessing the association of use of various contraceptive methods with ovarian cancer risk in New Zealand are presented and discussed.

CHAPTER 5: METHODS

5.1 STUDY DESIGN

This was a national population-based case-control study. A case-control study is an epidemiological study in which a group of people with the condition (cases) and a group without that condition (controls) are identified and the prevalence or level of the relevant exposure is measured in the two groups and compared. In this study, cases were women aged 35-69 years with recently diagnosed ovarian cancer and controls were women in the same age range without ovarian cancer. The prevalence of use, age at first use, time since last use, and duration of use of various contraceptive methods were compared between the two groups. Information about demographic characteristics and potential confounding factors was also collected.

A case-control study is well suited to study diseases of rare occurrence with long-induction periods, such as cancer, because no lengthy follow-up is involved [281]. The process of cancer initiation and promotion can last 20 or more years before a cancer becomes clinically detectable; however, because a case-control study starts with subjects who have already developed the disease, there is no need to wait for time to elapse between exposure and the occurrence of disease [236]. Instead of following up a very large group of women in order to identify the small proportion who develop ovarian cancer, all women who develop ovarian cancer within a short time period are eligible to be cases in the study [282]. A case-control study provides the possibility of investigating a wide range of possible risk factors.

There are a number of potential limitations of a case-control study. First, there is difficulty in determining the time-sequence between exposure and outcome because both are measured at the same time. This was not expected to affect our study because contraceptives are used by women in their reproductive years (prior to menopause), whereas ovarian cancer is a disease that mainly affects postmenopausal women. Second, odds ratios are used to estimate relative risks, which can result in different estimates. In this study, a significant difference was not expected between these two measures because ovarian cancer is a rare disease. Third, a case-control study is inefficient for rare exposures. Fourth, only one outcome can be assessed, which was not a limitation for this study because we were only interested in the development of ovarian cancer. The limitations of a case-control study (as affects this study) and steps taken to minimize these are discussed in Chapter 11 (section 11.2.2).

This design was suitable for this study because the controls are representative of the population from which the cases came, and because it is a national population-based study, the controls represent the general population of New Zealand women aged 35-69 years for the following exposures of interest: probability of ever having had a vasectomised partner and use of intrauterine contraceptive devices or long-acting progestogen-based contraceptives. In addition, despite ovarian cancer being a relatively rare condition in New Zealand, the study design allows for identification of adequate numbers of cases.

If cases and controls are selected independently of exposure and controls are randomly selected from disease-free members of a defined population from which the cases arose, a case-control study provides information that mirrors what could be learned from an equivalent cohort study, at less cost and time [281, 282]. Similar case-control studies of cancer have been carried out successfully in New Zealand [278, 283, 284].

5.2 STUDY AREA

The study was conducted in New Zealand (also known as Aotearoa) which has an area of 269,652 square kilometres, with a population of 4,242,048 according to the 2013 Census. The population has a diverse ethnic composition and individuals can have multiple ethnic affiliations [285]. In the 2013 Census the ethnic composition was: Europeans: 74%; Māori: 15%; Asian: 12%; Pacific peoples: 7%; and Middle Eastern/Latin American/African (MELAA): 1% [286] (percentages sum to >100% because people can identify with more than one ethnic group, and prioritised reporting is not used). English is an official language and also the most widely spoken language, being spoken by 90% of the population at the 2013 Census; the other official languages are Māori and New Zealand Sign Language [287].

Undertaking the case-control study in New Zealand was appropriate due to the presence of a well-developed healthcare system. New Zealand hospitals are well equipped and able to make histologic diagnosis of cancer before commencing treatment. In addition, there are statutory requirements for laboratories to report all cancer diagnoses (except non-melanoma skin cancer) to the New Zealand Cancer Registry (NZCR) and for all New Zealand citizens and permanent residents 18 years of age and above to register on the parliamentary electoral roll, facilitating recruitment of study subjects. Also of interest for the current study, New Zealand has a high prevalence of oral contraceptive use and vasectomy compared to other countries [288]. However, New Zealand's relatively small population meant that recruiting an adequate number of cases was a challenge.

The cases were obtained from the NZCR (and were also confirmed as being listed on the electoral roll), and the controls from the general population through the general and Māori electoral rolls.

5.3 STUDY POPULATION

The study population consisted of women with newly diagnosed, histologically confirmed ovarian cancer, aged 35 to 69 years, at any stage of disease but capable of effective communication in English. The controls consisted of women free from ovarian cancer with similar age restrictions and capable of effective communication in English.

Ovarian cancer is generally a disease of post-menopausal women, with the highest incidence at ages 65 to 74 years [23]. The mean age of menopause is 51.5 years [45]. Women use contraceptives during their reproductive years; that is, between menarche and menopause. The age range, 35 to 69 years inclusive, captures the population that is most affected by ovarian cancer and, at the same time, allows for best recall of contraceptive usage. To ensure that the controls represented the population from which the cases came, and that cases and controls could be contacted by mail, all eligible cases and controls were listed on the electoral roll.

5.4 INCLUSION AND EXCLUSION CRITERIA

Controls were randomly selected from the General and Māori Electoral Rolls. Cases were recruited sequentially from the NZCR and were eligible if they were also listed on the electoral rolls. Approval to approach the patients (cases) was sought from each woman's doctor. All participants gave informed consent to participate in the study and were capable of completing a self-administered or telephone-administered questionnaire. Women whose names were not listed on the electoral rolls were excluded from the study.

Approval from the doctors of the women who constitute the cases was sought with the assumption that each doctor knew the woman well and would, therefore, know whether she was well enough to participate in the study. To ensure that participants were at risk of a first primary ovarian cancer, women with a prior history of ovarian cancer were excluded. Controls with history of bilateral oophorectomy were also excluded because this effectively eliminates the risk of developing ovarian cancer.

Due to logistical constraints, potential participants who were unable to communicate in English were excluded. English being the most spoken language in New Zealand and

participants being New Zealand citizens or permanent residents, this requirement was not expected to exclude a significant number of potential participants.

5.5 SAMPLING

There was sequential recruitment of cases until the desired sample size was achieved. The controls were selected randomly from the electoral rolls, frequency matched to cases by 5-year age-groups. Histological reports of cases were reviewed in order to confirm the diagnosis of ovarian cancer, and approval was sought from the relevant doctor to approach each patient for participation in the study (a sample of the letter to doctors is provided as Appendix 2).

At the start of the study, a stratified random sample of controls was obtained from the electoral rolls. Data on incident cases of ovarian cancer for the years 2007, 2008, and 2009 were obtained from the NZCR and used as a basis for the number of controls to be recruited from each 5-year age group. After one year, adjustments were made according to the number of new ovarian cancer diagnoses in each age group, in order to ensure that the number of controls in each age-group was proportional to the cases. Participants were stratum-matched by age because the incidence of ovarian cancer increases with age. Samples of the letters to cases and controls, and the information sheet and consent form are provided as Appendices 3-6.

5.6 SAMPLE SIZE

In deciding on the sample size, it is important to ensure that the number of study participants is appropriate to answer the study objectives. A small study may fail to detect important effects on the outcome of interest, whereas a study larger than necessary wastes resources. The following were used to estimate an appropriate sample size for this study:

1. The power of the study (denoted by 1-β); that is, the probability that a study of a given size would detect a statistically significant real difference of a given magnitude, thus avoiding type II error (failing to find an association between the exposure of interest and occurrence of disease when an association exists). A power of 80% is considered acceptable.
2. Level of significance (α); that is, the probability of a type I error (finding an association between the exposure of interest and occurrence of disease when none exists). The usual value for α is 5%.

3. The magnitude of the anticipated strength of association (in this case an odds ratio of 0.5).
4. The prevalence of exposure to the different contraceptive methods among the controls.

At the start of the study, there were no recent data on the prevalence of use of the various contraceptive methods in New Zealand; the latest population-based studies were done in 1988 (for IUDs, vasectomy, and DMPA) [18] and 2001 for vasectomy [288]. Using the available prevalence data, it was determined that a sample size of 1,162 (291 cases and 871 controls) would provide 80% power with 95% confidence to detect an odds ratio of 0.5, with the prevalence of exposure to a given contraceptive method of 0.13 or higher. An odds ratio of 0.5 was determined by a combination of the association between use of different contraceptive methods and ovarian cancer reported in previous studies and an association that is strong enough to have clinical and public health relevance. To allow for non-response 1,396 women were to be approached: 1,047 controls and 349 cases.

The expected number of ovarian cancer cases per year in New Zealand, within the age range of 35 to 69 years inclusive is 175 [289]. Using the prevalence estimates of contraceptive use available at the beginning of the study, it was expected that, within 2.5 years, assuming a response proportion of 80%, we would have enrolled enough cases in the study to robustly estimate ovarian cancer risk for "ever-users" of long-acting progestogen-based contraceptives, IUDs and vasectomy. A response proportion of 80% was expected because recent New Zealand population-based case-control studies using the Cancer Registry and electoral rolls to identify participants, had response proportions of 80% to 88% for eligible cases and 74% to 80% for eligible controls [283, 284]. Factors affecting the response proportion in this study are discussed in section 11.2.2.2.

In order to confirm the prevalence estimates used, after the first year of the study the prevalence of exposure to the different contraceptive methods in the controls was calculated (prevalence estimates of contraceptive use are presented in Chapter 6). These prevalence data were used to refine the calculations on required sample size and hence adjust the length of the study. In view of the small number of ovarian cancer cases, more controls than cases were recruited (a case-control ratio of 1:5) in order to enhance the statistical power of the study (Table 5.1).

Table 5.1: Required sample sizes (Fleiss with continuity correction [CC]) assuming Odds Ratio of 0.5, significance level=0.05, power=0.8 and a case-control ratio of 1:5

Type of contraceptive	Prevalence of ever-use	Sample size		
		Cases	Controls	Total
Vasectomy	44.8%	95	472	567
IUDs	21.9%	164	818	982
DMPA	13.7%	256	1, 277	1,533

5.7 DATA-COLLECTION INSTRUMENT

The data were collected using a structured self-administered postal questionnaire (Appendix 7) covering the following areas:

1. Socio-demographic characteristics of the study participants: these included age, area of residence, country of birth, ethnicity, occupation, level of income, and level of education. These were to help judge whether the controls represented the population from which the cases arose, by comparing the frequency of these variables between the cases and controls and to identify any socio-demographic factors that were potential confounding factors.
2. Menstrual and reproductive history: this covered ages at menarche and at menopause, menopausal status, average menstrual cycle length and regularity, parity, duration of breastfeeding, and number of abortions. Other than being predictors of ovarian cancer, these data provided an estimate of the participants' reproductive window and ovulation years.
3. Family history of cancer: this covered the relationship of the affected family member to the participant, the type of cancer, and age at diagnosis. This provided insight to a possible genetic predisposition to ovarian cancer and allowed adjustment for possible confounding by family history.
4. History of contraceptive use: this included types of contraceptives used, age at first use, time since last use, and duration of use. This covered important data on use of the contraceptives being studied and other contraceptives that may also be potential confounders.
5. Potential confounding factors: these included both protective and risk factors for ovarian cancer.

The histopathology report for each case was provided by the NZCR. It included:

- date of birth;
- date of diagnosis (which provided the age of the patient at diagnosis);

- histologic type of cancer, which is important in confirming diagnosis and therefore, correct definition of cases;
- stage and grade of disease at diagnosis, which describes the severity of disease; and
- name of the treating doctor.

The questionnaire included previously validated questions (from the New Zealand census for demographic and other relevant information) and questions sourced from suitable questionnaires such as those used in the Million Women Study [142]. The use of questions used in the New Zealand 2013 census allowed comparison of the data obtained from this study with those from the census. A few questions were repeated to test the consistency of the answers given by the respondents. The questionnaire was pilot tested and revised before being used in the study.

There was no structured interview, but participants who did not wish to complete the questionnaire by mail were offered the option of completing the questionnaire by telephone, using the same questionnaire, carried out by a trained interviewer.

5.8 DATA-COLLECTION PROCEDURE

Recruitment of study subjects and data collection were done nationwide. Histology reports for all women with a recent diagnosis of ovarian cancer were forwarded to the research team from the NZCR (in accord with legislated provision for access to Cancer Registry data for health research). The reports were reviewed by a medically qualified member of the research team. All women with histologically confirmed diagnosis of ovarian cancer, at 35 to 69 years inclusive and who met the inclusion criteria for the study were selected. Approval to approach the patients about the study was then sought from each woman's relevant doctor (Appendix 2). Following approval from the doctors, those selected were sent a letter (Appendix 3) by mail inviting them to participate in the study. The letter was on University of Canterbury letterhead and was signed by two members of the research team. Each letter was accompanied by an information and consent form (Appendices 5 and 6), a copy of the questionnaire (Appendix 7), and a postage-paid addressed envelope for returning the questionnaire and signed consent form.

As described earlier, the controls were randomly selected from the New Zealand electoral rolls, frequency matched to cases by 5-year age-groups. Access to electronic data about people on the New Zealand Electoral roll for purposes of health research is allowed under section 112(3) of the Electoral Act 1993. Each potential control was sent a letter (Appendix

4) on University of Canterbury letterhead, signed by two members of the research team. The letter was accompanied by an information and consent form (Appendices 5 and 6), a copy of the questionnaire (Appendix 7), and a postage-paid addressed envelope for returning the questionnaire and signed consent form.

To facilitate responses, women who did not respond to the initial questionnaire and consent form within three weeks from the date of dispatch, were sent a second letter, information and consent form, questionnaire, and postage-paid addressed envelope. Women who did not respond to the second mail-out were contacted and asked to complete the questionnaire by telephone interview conducted by a trained interviewer. If they were willing to do this, a telephone interview was done, using the same questionnaire. They were requested to post back a signed consent form. Those who returned completed questionnaires without a signed consent form or who had a telephone interview and did not post back a signed consent form were considered to have consented. All questionnaires were checked for completeness; where necessary, participants were contacted to obtain missing data.

This approach has been used successfully in New Zealand population-based case-control studies using the Cancer Registry and electoral rolls, with response proportions of 80% to 88% for eligible cases and 74% to 80% for eligible controls [283, 284].

The data collection procedure is depicted in Figure 5.1.

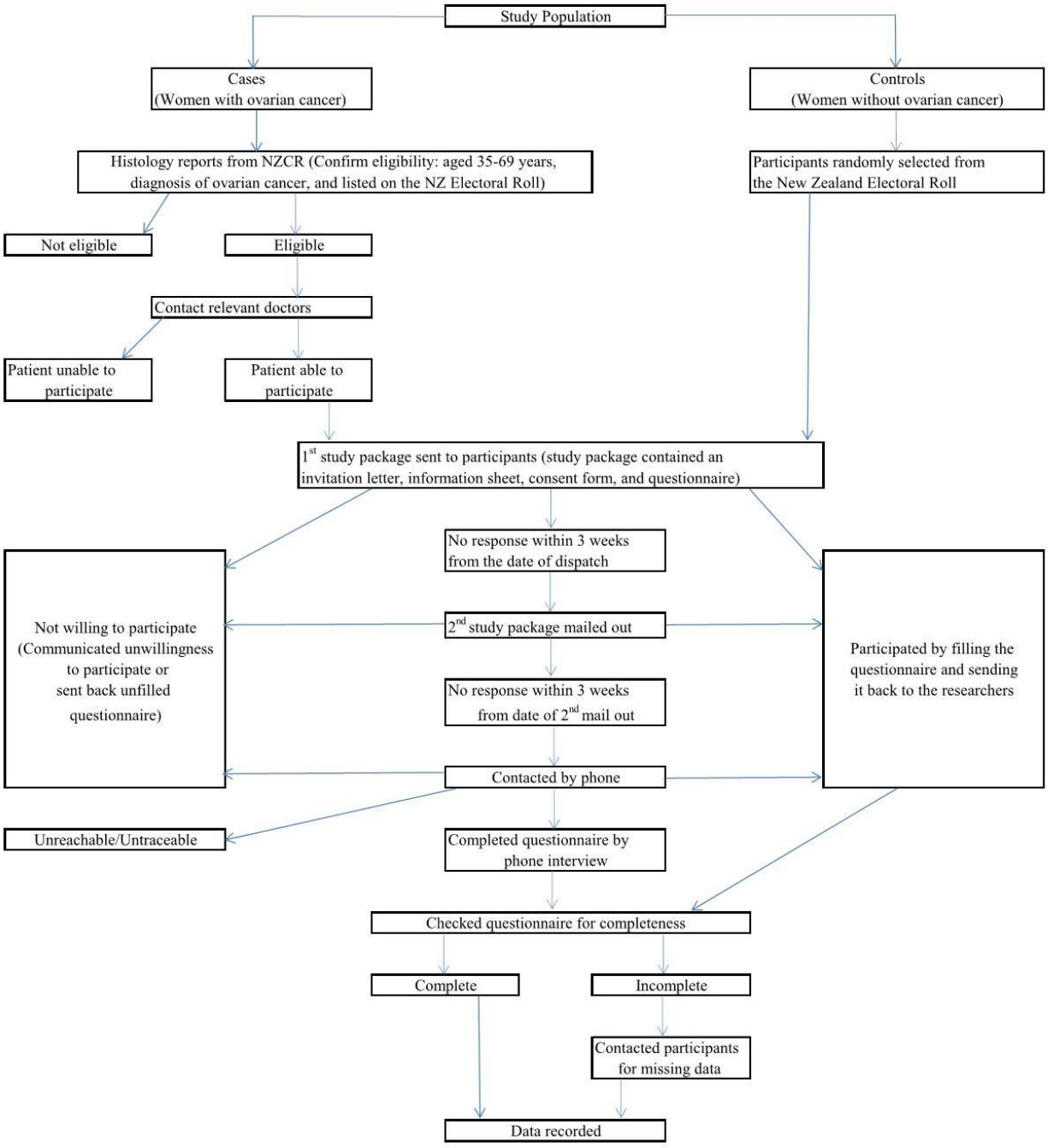


Figure 5.1: Data collection procedure

5.9 OUTCOME MEASURES

1. OR of ovarian cancer in ever-users of DMPA.
2. OR of ovarian cancer in ever-users of IUDs.
3. OR of ovarian cancer in relation to age at initiation of use, time since last use, and duration of use of the above contraceptives.
4. OR of ovarian cancer in women whose partner(s) have had vasectomy.

5. OR of ovarian cancer in relation to duration of reliance on vasectomy for contraception.

In a case-control study, if the prevalence of exposure among cases and controls is different, it is possible to infer that the exposure may be associated with an increased or decreased occurrence of the outcome of interest. The association is calculated in the form of an OR from the number of exposed cases (A), exposed controls (B), unexposed cases (C) and unexposed controls (D).

$$OR = \frac{(A/C)}{(B/D)} = \frac{AD}{BC}$$

The OR obtained from a case-control study, if it can be interpreted as an estimate of the relative risk, gives an indication of the likelihood of developing the disease in the exposed individual relative to those unexposed. In diseases of rare occurrence, the OR is approximately equal to the RR [290, 291]. Inference about the association between a disease and a factor is considerably strengthened if there is evidence of a gradient between the level of exposure and risk of the disease in question.

5.10 DATA MANAGEMENT AND ANALYSIS

The questionnaires were checked for completeness. An attempt to obtain missing data and to clarify discrepant values was made. Data were then entered into an EXCEL spreadsheet. Frequency tables were constructed in order to aid in identifying data entry errors. These errors were corrected by referring to the answers in the questionnaires. In situations where values provided were considered unrealistic (for example, a menstrual cycle length of less than 15 days), these were treated as missing values. All identifying information was coded before analysis.

Data were analysed using the IBM Statistical Package for the Social Sciences (IBM SPSS statistics 22). Descriptive statistics were used to compute frequencies and percentages. The chi-square test and t-test were used in the initial comparison of socio-demographic characteristics and predictors of ovarian cancer of cases versus controls (Appendix 12). All variables collected in the study were assessed for association with the risk of ovarian cancer, adjusted for age in 5-year groups (as cases and controls were not individually matched by age) using the method of Mantel and Haenszel [290]. When controlling for more than one variable, multivariate unconditional logistic regression was used. Odds ratios, 95%

confidence intervals, and p-values were reported. All statistical tests were 2-sided and the risk estimates were considered significant if the p-value was less than 0.05.

Ever-use and specifics of use of IUDs, vasectomy, and DMPA were also adjusted for:

- ever-use of oral contraceptives;
- ever-use of PMH;
- history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative;
- age at last delivery; and
- age in 5-year groups (the matching variable).

These factors were selected based on prior literature (Chapters 2 and 4) indicating their association with ovarian cancer. In addition, they had the strongest association with the risk of ovarian cancer and had a statistically significant independent association when entered together into the logistic regression model. History of cancer in a first-degree relative that suggested familial predisposition to ovarian cancer was associated with a p-value of 0.080 in age-adjusted analysis, but was nevertheless included in the multivariable regression model because of its known association with the risk of ovarian cancer.

Additional description of methods used, specific to Chapters 6-10, are included at the beginning of these chapters.

5.11 ETHICAL CONSIDERATIONS

No major ethical issues arose from this study as it involved no invasive procedures or treatment trials. Confidentiality was maintained at all levels. All identifying information was coded so that confidentiality was maintained. The research records were accessible only to the researchers and the research administrator who signed a confidentiality agreement. The data were stored on password-protected computers or filed in a lockable filing cabinet in a room which could be locked, in the University of Canterbury School of Health Sciences. There was no identification of individual participants during data analysis and dissemination. In recruiting cases, approval was sought from each patient's doctor to approach her for the study. Study numbers were inserted during printing of questionnaires and these were used in entering data. Potential participants were informed about the study, and informed consent was obtained before recruitment. Approval to conduct the study was obtained from the Southern Health and Disability Ethics Committee (13/STH/26) (Appendices 10 and 11) and the University of Canterbury Human Ethics Committee (HEC 2013/08) (Appendices 8 and 9).

We did not expect the participants to experience any mental stress or emotional distress as a result of this study. However, because the information that was being sought in this study was of a sensitive nature, the contact details of the Chair of the University of Canterbury Human Ethics Committee, and the Health and Disability Advocate were provided. Participants were also invited to ask the researchers questions about the study and to discuss the risks and/or benefits of the study with a friend, family or whānau. In addition, potential participants were informed that their participation was voluntary and they could withdraw from the study at any time without consequences.

The study hypothesis was withheld from the participants; this is important in a case-control study as participants' knowledge of the study hypothesis can potentially introduce information bias and therefore, affect the validity of the study and thus waste participants' time. Only the study hypothesis was withheld. This is accepted practice in case-control studies and is supported by "the International Ethical Guidelines for Epidemiological Studies" [292].

Only 13 Māori women with ovarian cancer took part in this study. This number was too small to allow separate analysis by ethnic group. However, the findings will be relevant to Māori women because ovarian cancer is the seventh most common cancer, and the fifth most common cause of death from cancer in New Zealand women. Age-standardised incidence and mortality rates for ovarian cancer are higher for Māori than for non-Māori women, but lower than for Pacific women [197]. It is not possible to reliably detect ovarian cancer early and not enough is known about ovarian cancer to prevent it. Increased understanding of the association between contraception and ovarian cancer may help to address this and limit the impact of this disease on Māori women and their whānau. The study proposal was assessed by the University of Canterbury Māori Research Advisory Group.

5.12 PARTICIPANTS' RESPONSE SUMMARY

The study was conducted from May 2013 through October 2015. For the control arm, a total of 1,903 New Zealand women aged 35-69 years at the time of selection (20/4/2013), were approached. Of these, 16 could not communicate in English, 8 were deceased, and 144 were no longer residing at the address indicated on the electoral roll. Of the remaining 1,735 women 837 participated in the study (response proportion of 48.2%), 413 were untraceable and 485 declined to participate. Reasons for declining to take part in the study were not

always given, but included participants being too busy to participate, involvement in another study, and finding some questions distressing or too personal.

Of the 837 women who participated, 81% (679) completed questionnaires and sent them back via post and 19% (158) were interviewed. Among the women who participated, 61 were excluded because they had a prior history of ovarian cancer diagnoses and/or bilateral oophorectomy or did not indicate their oophorectomy status and 30 were excluded because they were more than 69 years old at the time of completing the questionnaire. This left questionnaires from 746 women available for analysis.

A total of 258 histological reports of women with a diagnosis of incident invasive ovarian cancer were received from the NZCR. Of these, 34 were not listed on the New Zealand electoral roll (2 of these were also aged over 69 years); advice against contacting 6 of the participants was received from the treating doctor; 10 died before contact could be made, and 3 were not residing at the address indicated on the electoral roll and could not be located otherwise. Therefore, 205 case subjects were eligible and, of these, 152 (response proportion of 74.1%) took part, 42 declined to take part and, for 11 participants, contact could not be made. Of those who participated, 74% (112) sent in completed questionnaires and 26% (40) were interviewed. Reasons for declining to take part were not always given, but included being on chemotherapy, discomfort with questions asked, involvement in other studies, still coping with the diagnosis of cancer, and being too busy to participate. The average duration from diagnosis to receipt of histological reports by the researchers was 3.0 months (SD = 2.3), and the average time from diagnosis to participation was 5.1 months (SD = 2.4).

5.12.1 AGE DISTRIBUTION OF PARTICIPANTS

Table 5.2 shows the age distribution of cases and controls. There was no difference in age distribution between all cases received from NZCR and those who participated in the study (χ^2 = 3.041; df = 6; P = 0.804). The age distribution of cases and controls did not differ from what was expected at the beginning of the study (χ^2 = 6.856; df = 6; P = 0.334, and χ^2 = 8.356; df = 6; P = 0.213, respectively).

Table 5.2: Age distribution of cases and controls

Age[1] (Years)	2007-2009 (Expected)[2]	Cases From NZCR	Cases Participated	Eligible Controls Respondents
	No. (%)	*No. (%)*	*No. (%)*	*No. (%*
35-39	23 (4.6)	13 (5.1)	5 (3.3)	43 (5.8)
40-44	36 (7.3)	24 (9.4)	12 (7.9)	61 (8.2)
45-49	62 (12.5)	31 (12.1)	18 (11.8)	91 (12.2)
50-54	78 (15.7)	50 (19.5)	35 (23.0)	94 (12.6)
55-59	106 (21.4)	45 (17.6)	29 (19.1)	156 (20.9)
60-64	95 (19.2)	43 (16.8)	27 (17.8)	152 (20.4)
65-69	96 (19.4)	50 (19.5)	26 (17.1)	149 (20.0)
Total	496 (100)	256 (100)	152 (100)	746 (100)

[1]Reference age is defined as age at diagnosis for cases and age at completing the questionnaire for controls.
[2]Expected is based on age distribution of cases for the years 2007, 2008 & 2009 (numbers are total for the three years).

5.12.2 HISTOLOGICAL TYPES AND STAGE DISTRIBUTION OF OVARIAN TUMOURS

All cases had invasive ovarian cancer and all but three tumours were of epithelial type. The distribution of the histological types was as follows: serous, 59%; endometrioid, 19%; clear-cell, 9%; mucinous, 7%; and others, 7% (Table 5.3). This was representative of the histological distribution of all cases received from NZCR (χ^2 =7.075; df = 4; P = 0.132) and was also consistent with that observed in other studies elsewhere [26, 191, 192].

Table 5.3: Distribution of histological types of ovarian tumours among cases

Histological Type	Eligible pre-contact		Ineligible pre-contact	Total
	Participated	Not participated		
	No. (%)	*No. (%)*	*No. (%)*	*No. (%)*
Serous	90 (59)	30 (42)	12 (34)	132 (51)
Endometrioid	29 (19)	14 (20)	9 (26)	52 (20)
Clear cell	13 (9)	7 (10)	2 (6)	22 (9)
Mucinous	10 (7)	5 (7)	3 (9)	18 (7)
Others[1]	10 (7)	15 (21)	9 (26)	34 (13)
Total	152 (100)	71 (100)	35 (100)	258 (100)

[1]Others included mixed epithelial, undifferentiated, adenocarcinomas not otherwise stated, and non-epithelial tumours

A number of histological reports indicated the stage of ovarian cancer (Table 5.4). No material difference was observed between eligible respondents and non-respondents (χ^2 = 1.472; df = 3; P = 0.689), or between those who participated and all the reports received from NZCR (χ^2 = 0.521; df = 3; P = 0.914).

Table 5.4: Stage distribution of ovarian tumours among cases

Stage	Eligible pre-contact		Ineligible pre-contact	Total
	Participated	Not participated		
	No. (%)	*No. (%)*	*No. (%)*	*No. (%)*
I	30 (20)	9 (13)	6 (17)	45 (17)
II	16 (11)	7 (10)	2 (6)	25 (10)
III	19 (13)	9 (13)	6 (17)	34 (13)
IV	10 (7)	2 (3)	2 (6)	14 (5)
Not specified	77 (51)	44 (62)	19 (54)	140 (54)
Total	152 (100)	71 (100)	35 (100)	258 (100)

5.12.3 PARTICIPATION BY GEOGRAPHIC REGION

Postcodes were used to assess whether respondents and non-respondents differed by geographic region. New Zealand postcodes have four digits; the first digit, numbered 0-9, represents a geographic region (North to South) [293].

The geographic distribution of participants was significantly different from that of all potential participants contacted in the control series (χ^2 =21.974; df =9; P =0.009). This was due to low response proportion in the North Island compared to the South Island regions. However, when analysis was restricted to women with whom contact was achieved, no difference in response distribution was observed between all women located and respondents and non-respondents for both the case and control arms (cases: χ^2 =2.029; df = 9; P = 0.991, and χ^2 = 7.671; df = 9; P =0.568; controls: χ^2 =6.072; df = 9; P = 0.733, and χ^2 = 10.055; df = 9; P = 0.346). Distribution of participants and non-participants are shown in Tables 5.5 and 5.6.

Table 5.5: Response distribution of all participants and non-participants across the 10 geographic regions

Post Code[1]	Controls			Cases (eligible pre-contact)		
	Participants[2] (Eligible and ineligible)	Non-Participants (All)	Total	Participants	Non-participants	Total
	No. (%)	*No. (%)*	*No. (%)*	*No. (%)*	*No. (%)*	*No. (%)*
0-999	112 (13)	190 (18)	302 (16)	18 (12)	12 (17)	30 (13)
1000-1999	76 (9)	111 (10)	187 (10)	13 (9)	10 (14)	23 (10)
2000-2999	57 (7)	126 (12)	183 (10)	13 (9)	4 (6)	17 (8)
3000-3999	162 (19)	173 (16)	335 (18)	24 (16)	7 (10)	33 (15)
4000-4999	97 (12)	127 (12)	224 (12)	24 (16)	6 (8)	30 (13)
5000-5999	67 (8)	80 (8)	147 (8)	11 (7)	7 (10)	18 (8)
6000-6999	25 (3)	35 (3)	60 (3)	8 (5)	4 (6)	12 (5)
7000-7999	99 (12)	95 (9)	194 (10)	12 (8)	7 (10)	19 (9)
8000-8999	71 (8)	66 (6)	137 (7)	10 (7)	7 (10)	17 (8)
9000-9999	71 (8)	63 (6)	134 (7)	19 (13)	5 (7)	24 (11)
Total	837 (100)	1066 (100)	1903 (100)	152 (100)	71 (100)	223 (100)

[1]0-9, represents a geographic region (North to South)

[2]Participants (eligible and ineligible) include all those who took part in the study including those who were later excluded because they were more than 69 years of age at the time of completing the questionnaire, or had prior diagnosis of ovarian cancer or had undergone bilateral oophorectomy.

Table 5.6: Response distribution of participants and non-participants across the 10 geographic regions restricted to those with whom contact was achieved

Post Code[1]	Controls			Cases (eligible post-contact)		
	Participants[2] (Eligible and ineligible)	Non-Participants[3] (Contact achieved)	Total	Accepted	Declined	Total
	No. (%)	*No. (%)*	*No. (%)*	*No. (%)*	*No. (%)*	*No. (%)*
0-999	112 (13)	86 (17)	198 (15)	18 (12)	6 (14)	24 (12)
1000-1999	76 (9)	42 (8)	118 (9)	13 (9)	7 (17)	20 (10)
2000-2999	57 (7)	48 (9)	105 (8)	13 (9)	1 (2)	14 (7)
3000-3999	162 (19)	78 (15)	240 (18)	24 (16)	4 (10)	28 (14)
4000-4999	97 (12)	76 (15)	173 (13)	24 (16)	3 (7)	27 (14)
5000-5999	67 (8)	34 (7)	101 (8)	11 (7)	5 (12)	16 (8)
6000-6999	25 (3)	17 (3)	42 (3)	8 (5)	3 (7)	11 (6)
7000-7999	99 (12)	55 (11)	154 (11)	12 (8)	6 (14)	18 (9)
8000-8999	71 (8)	31 (6)	102 (8)	10 (7)	3 (7)	13 (7)
9000-9999	71 (8)	42 (8)	113 (8)	19 (13)	4 (10)	23 (12)
Total	837 (100)	509 (100)	1346 (100)	152 (100)	42 (100)	194 (100)

[1]0-9, represents a geographic region (North to South)

[2]Participants (eligible and ineligible) include all those who took part in the study including those who were later excluded because they were more than 69 years of age at the time of completing the questionnaire, or had prior diagnosis of ovarian cancer or had undergone bilateral oophorectomy.

[3]Among non-participants, contact achieved included those who declined to take part, deceased, and those who were unable to communicate in English, while contact not achieved included those who were not at the addresses indicated on the electoral roll and those who could not be traced.

The findings of this study are presented in Chapters 6 to 10.

CHAPTER 6: PREVALENCE OF CONTRACEPTIVE USE IN NEW ZEALAND WOMEN

This chapter has been submitted for publication.

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6.1 ABSTRACT

AIMS: To estimate the prevalence of contraceptive use among New Zealand women and to measure changes in contraceptive use since the last population-based prevalence estimates were published in 1988.

METHODS: 904 women, aged 35-69 years were randomly selected from the electoral roll. A postal questionnaire was used to gather information on contraceptive use, socio-demographic characteristics, and risk factors for ovarian cancer. Data were collected in 2013-2015. Estimates of current and ever-use of contraceptives were made and compared with the findings of the 1988 study by Paul et al. In both studies, participants were members of the control arm of case-control studies.

RESULTS: The study by Paul et al. had a response proportion of 84%, whereas that of the current study was 47%. Oral contraceptives had the highest prevalence of ever-use among women aged 35-69 years (89% [347/389]), followed by condom use (54% [211/389]), and vasectomy (44% [170/389]). Compared to the previous study, among women aged 35 to 54 years, there has been an increase in ever-use of condoms (24% [185/767] to 64% [148/231]), vasectomy (26% [202/767] to 40% [92/231]), and oral contraceptives (75% [575/767] to 89% [205/231]). In contrast, a lower prevalence of tubal ligation (22% [168/767] to 8% [19/231]) was observed.

CONCLUSION: The study demonstrates a change in patterns of contraceptive use among women aged 35-54 years. The prevalence of ever-use of oral contraceptives and vasectomy remains high in New Zealand compared with other countries.

Since the introduction of oral contraceptives (OCs) in the 1960s [294], there have been substantial advances in the development of contraceptive methods, including transition from

high-dose to low-dose OCs, and from inert to copper-bearing and levonorgestrel-releasing intrauterine contraceptive devices [17]. Currently, there is a wide range of safe and effective contraceptive methods available. From a public health perspective, up-to-date knowledge of patterns of contraceptive use is important, as contraceptives exert effects that could be beneficial or harmful to some users.

There has also been a significant change in age at first delivery among New Zealand women, which may have affected patterns of contraceptive use. Among women born in the 1960s, 42% had their first child before the age of 25 years, compared with 60% of women born before 1950 [295]. In 1962, women in their twenties had the highest fertility rates, while in 2014 the highest fertility rates were in women in their thirties. These changes were accompanied by a decrease in fertility rates across all age groups [296].

A population-based study on patterns of contraceptive use in New Zealand by Paul et al. was published in 1988 [18]. Women aged 25-54 years, were randomly selected from the New Zealand electoral roll. The participants, who were part of the control arm of a population-based case-control study of breast cancer and hormonal contraception, were recruited during 1983 to 1986. A more recent study [297], which recruited participants from North Waikato and Auckland City only, was restricted to women who had ever had sexual intercourse, and did not report age-specific contraceptive use.

The estimates reported in this paper are derived from a recent population-based case-control study designed to assess the association between ovarian cancer and use of contraceptives. The availability of information on contraceptive use among controls offered an opportunity to assess current and recent contraceptive practice in women over 35 years of age. Current patterns of contraceptive use in New Zealand women aged 35-69 years are presented, and comparisons with the previous population-based study for women aged 35-54 years (the common age range), are made.

6.2 METHODS

Study participants were members of the control arm of a nationwide population-based case-control study on the association between contraceptive use and ovarian cancer. Women were recruited into this study between May 2013 and September 2015. A random sample stratified by 5 year age-groups of women aged 35 to 69 years was obtained from the electoral roll. All New Zealand citizens and permanent residents 18 years of age and above are required by law to register on the electoral roll. Access to electronic data from the electoral roll for the

purposes of health research is allowed under section 112(3) of the Electoral Act 1993. The choice of the age limits 35 to 69 years was constrained by the ovarian cancer and contraception study. Ovarian cancer is generally a disease of post-menopausal women, with the highest incidence at ages 65 to 74 years. The age range 35 to 69 years includes the population that is most affected by ovarian cancer and at the same time allows for recall of contraceptive usage. Approval to conduct the study was obtained from the Southern Health and Disability Ethics Committee (13/STH/26) and the University of Canterbury Human Ethics Committee (HEC2013/08).

Each potential participant was sent a letter on University of Canterbury letterhead, signed by two members of the research team. The letter was accompanied by an information and consent form, a copy of the study questionnaire, and a post-paid addressed envelope for returning the questionnaire and signed consent form. To facilitate the responses, women who did not respond to the initial questionnaire and consent form within three weeks from the date of dispatch, were sent a second study pack. Women who did not respond to the second mail-out were contacted and asked to complete the questionnaire by telephone. If they were willing to do this, a telephone interview was done, using the same questionnaire. All questionnaires were checked for completeness; where necessary, participants were contacted to obtain missing data.

Participants were asked about ever-use, age at first use, time since last use and duration of use of oral contraceptives, DMPA, contraceptive implants, and IUDs. History of and age (if applicable) at menopause, hysterectomy, tubal ligation and bilateral oophorectomy were also asked. Information on ever-use and duration of reliance on condoms and vasectomy or other contraceptives was sought. In addition, information on socio-demographic characteristics of the participants and risk factors for ovarian cancer was also gathered. Participants were provided with a calendar of major life events to assist in recall and to record their use of contraceptives.

Age of participants was calculated in two ways. For the purpose of comparing with the New Zealand 2013 census population, age was calculated as the difference between each participant's date of birth and the date she was selected from the electoral roll. For prevalence estimations, the difference between date of birth and date of questionnaire completion was used to calculate age. Level of education was classified by the highest qualification attained using the 2013 census categories. Income was based on the total personal pre-tax income in

the last year. Menopause was defined as the age periods stopped, women with natural menopause and those with iatrogenic menopause were classified as postmenopausal. Analysis of current contraceptive use was restricted to women aged 35-54 years. This is because those above 54 years were most probably postmenopausal and would therefore have no need for contraception. Those who were postmenopausal but within 35-54 years of age were included as currently not using a reversible contraceptive method in order to account for all the participants.

In the previous population-based study by Paul et al. [18] study participants were members of the control arm of a nationwide population-based case-control study of breast cancer and hormonal contraception. Women aged 25-54 years were randomly selected from the New Zealand electoral rolls. Only those with traceable telephone numbers were included. Recruitment of participants was done from 1st November, 1983 to 5th February, 1986, and a response proportion of 84% was achieved.

The study by Paul et al.[18] included women aged 25-54 years, whereas the current study included women aged 35-69 years. In comparing the two studies, the prevalence estimates were restricted to women aged 35-54 years, the overlapping age-range for the two studies. In addition, proportions were weighted to account for the age structure of both samples. The age-groups used in comparing the two studies are similar to those used in the publication of the study by Paul et al. The lead author was also contacted to verify our accuracy in data extraction from their publication. In both studies, the purpose of examining ethnic groups was to compare the participants with the census population. In the study by Paul et al., prioritised ethnicity was used because this was used at that time by Statistics New Zealand. At the time of our study, prioritised ethnicity was no longer used by Statistics New Zealand. Therefore, in our study, ethnicity was classified according to the 2013 census categories. This was appropriate because we needed to compare the ethnic distribution of the participants with that of similarly aged New Zealand women in order to assess whether they were a representative sample.

Data were analysed using the IBM Statistical Package for the Social Sciences (IBM SPSS statistics 22). Descriptive statistics were used to compute frequencies and percentages. The chi-square test was used to examine the associations between socio-demographic characteristics of the participants and the female usually resident population at the 2013 census. Differences in the age standardised prevalence of contraceptive use between the two

studies and 95% confidence intervals were estimated in order to assess statistical significance.

6.3 RESULTS

There were 904 women selected from the electoral roll. Of these, 59 were not currently residing at the address indicated on the electoral roll and 15 could not communicate in English. Of the remaining 830 women, 255 declined to participate and 184 could not be located. This left 389 women who were available for the analysis (response proportion = 47%).

The age profile of the sample population was representative of the 2013 census female population aged 35-69 years. Although participants had a higher level of education than the general population (χ^2 = 58.455, df =6, P <0.001), other socio-demographic characteristics of the participants were comparable to those of the female usually resident population aged 35-69 years (Table 6.1). When response by geographic region was assessed, a higher response proportion was noted in the South Island compared to the North Island. However, when only participants where contact was achieved were considered, excluding those who could not be located, participation was equal across regions.

Table 6.1: Socio-demographic characteristics of women in the present study and the female usually resident population aged 35-69 years- 2013 Census

Characteristic	Women in present study		Female usually resident population -2013 Census	
	Number	(%)[1]	Number[2]	(%)[1]
Age (Years)	***(n = 389)***		***(n = 969,111)***	
35-44	111	(29)	302,835	(31)
45-54	120	(31)	312,723	(32)
55-64	101	(26)	253,089	(26)
65-69	57	(14)	100,464	(10)
Ethnicity[3]	***(n=389)***		***(n= 921,423)***	
NZ European[4]	316	(81)	714,276	(78)
Māori	37	(10)	111,828	(12)
Others[5]	58	(15)	-	-
Parity	***(n=327)***		***(n = 783,810)***	
0	47	(14)	120,867	(15)
1	32	(10)	108,414	(14)
2	144	(44)	283,707	(36)
3	66	(20)	166,608	(21)
4	25	(8)	64,860	(8)
5	10	(3)	22,470	(3)
6 and Over	3	(1)	16,884	(2)
Education[6]	***(n=321)***		***(n = 792,375)***	
No qualification	34	(11)	138,987	(18)
Overseas Secondary School Qualification	12	(4)	60,657	(8)
Level 1 or 2 Certificate	76	(24)	202,962	(26)
Level 3 or 4 Certificate	40	(13)	99,240	(13)
Level 5 or 6 diploma	57	(18)	93,669	(12)
Bachelors degree and level 7 qualification	70	(22)	129,564	(16)
Postgraduate[7]	32	(10)	67,296	(8)

[1]Percentages are of total stated.
[2]The total numbers of female usually resident population in the 4 categories differ because of values that were not stated.
[3]Some participants identified with more than one ethnicity, hence > 100%.
[4]NZ European includes those who identify themselves as 'New Zealanders' as this option was not provided in the study questionnaire.
[5]'Others' could not be computed from the NZ 2013 census results
[6]The estimates presented for parity and education are limited to 35 to 64 year old women. This is because the census data does not provide the level of education and parity for the 65-69 years age-group; instead this is given as 65 years and over.
[7]Postgraduate includes postgraduate honours degree, Masters degree, and Doctorate.

6.3.1 CONTRACEPTIVE EVER-USE

The results of contraceptive ever-use are presented in Table 6.2. Oral contraceptives had the highest prevalence of use (89%), with almost uniform use across age-groups. This was followed by condom use (54%), with the proportion of users lower at higher ages. Implants had the lowest prevalence (1%); only 4 of the 389 women had used an implant. Overall, ever-

use of reversible contraceptives declined with age. In contrast, prevalence of vasectomy, tubal ligation, and hysterectomy increased with age. No participant had undergone tubal ligation reversal operation. More than half (52%) of the participants were post-menopausal.

Table 6.2: Proportion of women who have ever-used various contraceptive methods according to age

	35-44		45-54		55-64		65-69		Total	
	No.	(%)[1]	No.	(%)[1]	No.	(%)[1]	No.	(%)[1]	No.	(%)[1]
Contraceptive Type[2]										
Pills	86	(77)	119	(99)	91	(90)	51	(89)	347	(89)
DMPA[3]	19	(17)	16	(13)	11	(11)	7	(12)	53	(14)
Implants	3	(3)	1	(1)	-	-	-	-	4	(1)
IUDs[4]	17	(15)	30	(25)	27	(27)	11	(19)	85	(22)
Condoms	72	(65)	76	(63)	46	(46)	17	(30)	211	(54)
Other[5]	3	(3)	16	(13)	8	(8)	7	(12)	34	(9)
Tubal ligation	3	(3)	16	(13)	22	(22)	21	(37)	62	(16)
Vasectomy	27	(24)	65	(54)	51	(50)	27	(47)	170	(44)
Bilateral oophorectomy	-	-	4	(3)	8	(8)	5	(9)	17	(4)
Hysterectomy	2	(2)	19	(16)	21	(21)	20	(35)	62	(16)
Periods Stopped[6]	6	(5)	48	(40)	88	(87)	53	(93)	195	(50)
Total number of women	111	(100)	120	(100)	101	(100)	57	(100)	389	(100)

[1]Percentages weighted to account for the age structure of the sample
[2]Some women used more than one method.
[3]Depot medroxyprogesterone acetate
[4]Intrauterine contraceptive devices
[5]Other included- diaphragm, cervical cap, natural method, chemical methods, and emergency contraceptive pills
[6]Includes natural menopause and iatrogenic menopause

6.3.2 CURRENT CONTRACEPTIVE USE

Of the 389 women who were available for analysis, 231 were aged 35-54 years. The results of the women currently practicing contraception are presented in Table 6.3. The method with the highest prevalence of current use was the oral contraceptives (9%), closely followed by the IUD (8%). As in ever-use, implants had the lowest prevalence of current use. An inverse relationship between age and prevalence of current use of reversible contraceptive methods was seen.

Among non-users of reversible contraceptive methods, 58% (101/175) were either sterilised (TL/hysterectomy) or have, at some point, had a vasectomised partner (participants were not asked about current use of vasectomy therefore this cannot be reported). However, 13 women who were either sterilised or had a history of a relationship with a vasectomised partner were also on a reversible method of contraception.

Table 6.3: Proportion of women on different contraceptive types according to age.

	35-39 No. (%)[1]	40-44 No. (%)[1]	45-49 No. (%)[1]	50-54 No. (%)[1]	Total No. (%)[1]
Using a reversible method					
Pills	5 (11)	10 (15)	3 (5)	3	21 (9)
DMPA[2]	- -	2 (3)	1 (2)	- -	3 (1)
Implants	- -	1 (2)	1 (2)	- -	2 (1)
IUDs[3]	3 (7)	9 (14)	6 (10)	1	19 (8)
Not using a reversible method[4]					
Vasectomy	8 (17)	14 (22)	30 (48)	29 (50)	81 (35)
TL[5]	- -	3 (5)	7 (11)	7 (12)	17 (7)
Hysterectomy	- -	2 (3)	4 (6)	15 (26)	21 (9)
Bilateral Oophorectomy	- -	- -	1 (2)	3 (5)	4 (2)
Period Stopped[6]	- -	3 (5)	9 (15)	35 (60)	47 (20)
Total number of women	46 (100)	65 (100)	62 (100)	58 (100)	231 (100)

[1]Percentages weighted to account for the age structure of the sample
[2]Depot medroxyprogesterone acetate
[3]Intrauterine contraceptive devices
[4]Although it is possible for vasectomy and TL to be reversed, this rarely occurs, so they have been classified as not reversible.
[5]Tubal ligation
[6]Includes natural menopause and iatrogenic menopause

6.3.3 COMPARISON WITH THE 1983-86 ESTIMATES OF CONTRACEPTIVE USE

6.3.3.1 Contraceptive ever-use

In the study by Paul et al. [18], 767 women were aged 35-54 years; there were 231 in the current study. Among women aged 35 to 54 years, over a span of 30 years, ever-use of condoms has more than doubled (24% to 64%). Increases in ever-use of OCs (75% to 89%), vasectomy (26% to 40%), and DMPA (10% to 15%) were also observed. In contrast, the prevalence of tubal ligation declined from 22% to 8%. The increase in reversible contraceptive use was uniform across age-groups apart from IUDs in which a decline in ever-use was observed in the younger age-group (35 to 44 years). The fall in tubal ligation was largely due to lower prevalence among younger women (Table 6.4).

Differences in age standardised prevalence between the current and previous study were statistically significant for oral contraceptives (15.0%; 95% CI = 10.1-19.9), condoms (39.4%; 95% CI = 32.5-46.3), tubal ligation (13.0%; 95% CI = 8.5-17.5), and vasectomy (13.8%; 95% CI = 7.1-20.6). There was no significant change in use of DMPA (5.0%; 95% CI = -0.1-10.1) or IUDs (4.0%; 95% CI = -1.7-9.8).

Table 6.4: Comparison of contraceptive ever-use between the present and previous study in women aged 35-54 years

	Paul et al.: (1983-1986)[18]			***Current Study (2013-2015)***		
	35-44	45-54	Total	35-44	45-54	Total
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Type of contraception						
Pill	352 (86)	223 (62)	575 (75)	86 (77)	119 (99)	205 (89)
Injection	52 (13)	28 (8)	80 (10)	19 (17)	16 (13)	35 (15)
IUDs	88 (22)	41 (11)	129 (17)	17 (15)	30 (25)	47 (20)
Condom	78 (19)	107 (30)	185 (24)	72 (65)	76 (63)	148 (64)
Tubal Ligation	115 (28)	53 (15)	168 (22)	3 (3)	16 (13)	19 (8)
Vasectomy	120 (29)	82 (23)	202 (26)	27 (24)	65 (54)	92 (40)
Total number of women	408 (100)	359 (100)	767 (100)	111 (100)	120 (100)	231 (100)

Percentages weighted to account for the age structure of the sample
Some women had used more than one contraceptive.

6.3.3.2 Current contraceptive use

In both studies the most common currently used contraceptives were pills, followed by IUDs, and DMPA, albeit with an increase in the proportion of users from 5% to 9%, 3% to 8%, and 0.3% to 1% respectively. In addition, a fall in female sterilisation and a rise in vasectomy were observed. In both studies, there was a consistent pattern of a higher prevalence of current use of reversible contraceptive methods among the 35-44 year olds compared to the 45 to 54 year olds. Overall, the prevalence of current use of reversible contraceptive methods was similar in both studies; 19% and 20%. The results are presented in Table 6.5.

In contrast to ever-use, the difference in age standardised prevalence of current use between the current and previous study was not statistically significant for oral contraceptives (4.6%; 95% CI = 0.6-8.5), but was statistically significant for IUDs (5.1%; 95% CI = 1.3-8.8). Similar to ever-use, the change in use of DMPA was not statistically significant (1.0%; 95% CI = -0.5-2.5).

Paul et al. [18] reported differences in contraceptive use according to socio-economic groups. However, in the present study no association between contraceptive use and income levels was observed, nor was there any relationship with level of education (data not shown).

Table 6.5: Comparison of prevalence of current contraceptive use between the present and previous study in women aged 35-54 years

	Paul et al.: (1983-1986) [18]			***Current Study (2013-2015)***		
	35-44	45-54	Total	35-44	45-54	Total
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Using a reversible method						
Pills	26 (6)	10 (3)	36 (5)	15 (14)	6 (5)	21 (9)
DMPA injection	1 (0.2)	1 (0.3)	2 (0.3)	2 (2)	1 (1)	3 (1)
IUD	17 (4)	8 (2)	25 (3)	12 (11)	7 (6)	19 (8)
Not using a reversible method						
Sterilised						
Tubal Ligation	101 (25)	33 (9)	134 (17)	3 (3)	14 (12)	17 (7)
Vasectomy	103 (25)	50 (14)	153 (20)	22 (20)	59 (49)	81 (35)
Hysterectomy	64 (16)	75 (21)	139 (18)	2 (2)	19 (16)	21 (9)
Postmenopausal	66 (16)	185 (52)	251 (33)	3 (3)	44 (37)	47 (20)
Total number of women	408 (100)	359 (100)	767 (100)	111 (100)	120 (100)	231 (100)

Some women used more than one contraceptive method concurrently
Percentages weighted to account for the age structure of the sample
Although it is possible for vasectomy and tubal ligation to be reversed, this rarely occurs, so they have been classified as not reversible.

6.4 DISCUSSION

In this population-based study of women aged 35-69 years, OCs had the highest proportion of ever-use (89%), followed by condom use (54%); implants had the lowest prevalence (1%). The prevalence of vasectomy, tubal ligation, and hysterectomy showed a positive relationship with age. In contrast to ever-use, the most common currently used contraceptives were IUDs and OCs. Implants had the lowest prevalence of current use.

Estimates of prevalence of contraceptive use, restricted to women aged 35-54 years, were compared with the previous study [18]. A significant rise in ever-use of the pill (75% to 89%) and a more than twofold increase in condom use (24% to 64%) were observed. There was a significant increase in ever-use of vasectomy (26% to 40%), accompanied by a fall in tubal ligation (22% to 8%). Only a slight increase in the use of DMPA and IUDs was observed.

Of participants currently not using reversible contraceptives, 58% had a tubal ligation or hysterectomy, or had, at some point, had a vasectomised partner. This may explain their non-use of reversible contraceptives. However, 13 women in this group were also on a reversible method of contraception. A subsequent change of partner or the use of contraceptives for non-contraceptive reasons may explain this.

In a report on current contraceptive use in New Zealand women aged 35-49 years, a fall in sterilisation (both male and female sterilisation) and a rise in condom use were observed during 1976 to 2001, the latter being consistent with the current findings. Use of oral contraceptives remained relatively constant during this period. In contrast, a fall in current

use of IUDs in younger women (35-39 years) and a rise in older women (40-49 years) were observed [298].

The previous study [18] reported a positive association between current contraceptive use and socio-economic status. In the current study no relationship was observed between current use and income or level of education. The difference in findings may be attributed to a change in contraceptive use, or to use of different measures of socio-economic status. Paul et al. [18] used the Elley-Irving scale for categorising social class. This method uses occupation as the main determinant of social class and is no longer in use. Furthermore, for married women the occupation of the husband was taken into consideration. In contrast, we used each participant's own annual income and highest level of education.

Similar to other studies [294, 299, 300], oral contraceptives had the highest prevalence of ever-use. This may be because oral contraceptives have been in use for longer than some other methods, and that they are also used for the management of some medical conditions. In addition, a high level of satisfaction among users of oral contraceptives has been reported [300] and they may be more acceptable than DMPA which has been available for almost the same duration [301]. Contraceptive implants were only recently introduced [299] and this may partly explain the low uptake seen in the present study. The need for special training in insertion techniques, affordability, side-effects, and awareness of availability of the method are additional factors that may contribute to low prevalence of use, however reasons for discontinuation or choice of a method were not sought in this study. Choice of method of contraception may also be influenced by health policy and funding such that changes in such policies may lead to changes in the prevalence of use of certain contraceptives.

The observed increase in the use of condoms has also been reported in other studies [294, 302]. A US study attributed the increase in women currently using contraceptives from 56% in 1982 to 64% in 1995 to a rise in condom use [302]. With the advent of HIV/AIDs, public education has promoted the use of condoms as a way of reducing the risk of sexually transmitted infections; this may explain the increase in use of condoms [294, 300]. The use of condoms in our study may have been underreported because participants were asked about condom ever-use as a contraceptive and not as protection against sexually transmitted infections. Indeed, studies have reported a higher prevalence of condom use in combination with other contraceptives as compared to use of condoms as the primary method; one study reported a rise from 20% to 23%, and another from 16% to 25% [300, 302].

Over 30 years in New Zealand, an increase in ever-use of vasectomy (26% to 40%) was observed. In a study conducted during 1997 to 1999, men aged 40-74 years were asked about their personal history of vasectomy; a prevalence of 44% was reported [288]. The prevalence of vasectomy may have been under reported in our study because women may not be aware of the vasectomy status of their partners. The increase in prevalence of sterilisation with increasing age is expected because younger women may still desire to have children.

From the findings of the current study, the prevalence of use of permanent methods of contraception (vasectomy and tubal ligation) in New Zealand has not changed in the last 30 years. What has changed is a couple's choice of sterilisation procedure, such that with the fall in the prevalence of tubal ligation there is a compensatory rise in the prevalence of ever-use of vasectomy. The shift to vasectomy may be due to ease of performing the procedure, lower risk of complications, and change in men's attitude towards sterilisation.

A decline in hysterectomy by half (18% to 9%) in women aged 35 to 54 years was observed. This is consistent with estimates of the prevalence of hysterectomy made for setting outcome targets for the New Zealand National Cervical Screening Programme [303].

Strengths of this study are the nationwide population-based design, and that it is representative of the age-distribution and ethnicity of New Zealand women in the 35 to 69 years age-range. Some potential limitations of this study should be considered. The low response proportion (47%) may have introduced selection bias. Women with higher levels of education were over-represented in this study, but response did not differ by age-group. When response by geographic region was assessed, a higher response proportion was noted in the South Island compared to the North Island. However, when only participants where contact was achieved were considered, excluding those who could not be located, participation was equal across regions. This difference may also be explained by high population mobility in the North Island. The Auckland region has the highest proportion of people who change residences between censuses, with some areas having only 10% of the population living at the same address between the 2001 and 2006 censuses [296]. Similar studies in New Zealand in the 1980s [18] and 1990s [288] had higher response proportions (84% and 85% respectively). In contrast to the current study, the inclusion criteria for those studies required participants to have traceable telephone numbers. Household access to a landline telephone has decreased in New Zealand accompanied by a rise in the use of cell phones [304].

The study relied on self-reported exposures which were not verified by medical records. However, in previous New Zealand studies in which corroborative information was obtained from medical practitioners, information on contraceptive use provided by the participants was consistent with that on their medical records [18, 288]. In addition, it is expected that participants may forget patterns of use of a particular contraceptive (age of onset, duration of use and last age), but not the type of contraceptive used.

The changes observed in contraceptive practice in New Zealand are a marked increase in the prevalence of vasectomy and condom use, and a slight increase in ever-use of oral contraceptives. In contrast, there was a fall in female sterilisation. New Zealand has a high prevalence of vasectomy and ever-use of oral contraceptives compared to other countries [294, 299, 300]. For example, in 2003, among women aged 15-49 years in 5 European countries (France, Germany, Italy, Spain, and United Kingdom), 85% were ever-users of oral contraceptives and 11% used sterilisation methods (both female and male sterilisation) [300]. Patterns of contraceptive use may be changing; therefore monitoring is required in order to meet the contraceptive needs of the New Zealand population. Knowledge of this may influence public health policy. The use of permanent sterilisation and long-acting reversible contraceptives (LARCs) affect the rate of unintended pregnancy and abortion rates, which are of public health importance. Population-based estimates of the prevalence of contraceptive use are also useful for calculating population attributable fractions for diseases related to contraceptive use [305].

CHAPTER 7: RISK FACTORS FOR OVARIAN CANCER

7.1 INTRODUCTION

In this chapter, the results of the analysis of risk factors – those associated with either higher or lower risk - for ovarian cancer will be presented and discussed in order to achieve three goals. First, other than young age, use of oral contraceptives, parity, and breastfeeding, which have been consistently associated with a lower risk of developing ovarian cancer, an association between ovarian cancer and other factors is unclear (See Chapter 2 - section 2.5). The findings of this Chapter will add to knowledge on ovarian cancer. Secondly, analysis of the influence of other variables is needed in order to identify factors that have an independent association with the risk of ovarian cancer and with the exposures of greatest interest in this study, and are not part of the causal pathway i.e., possible confounders. The third reason is for comparison with the findings of other studies. Results consistent with those of other studies may support causal associations (by fulfilling the Bradford Hill criterion of consistency [144]) and provide evidence that our study methods are valid.

A summary of participants and discussion of methods have already been provided in Chapter 5, and information on the prevalence of contraceptive use in New Zealand women was provided in Chapter 6. This chapter presents the results of an analysis of risk factors for ovarian cancer, whereas Chapters 8, 9, and 10 will present the results of analyses of ovarian cancer and DMPA, IUDs, and vasectomy, respectively.

7.2 METHODS

The data were collected using a structured self-administered postal questionnaire (Appendix 7). For more details on the data-collection instrument and procedure refer to Chapter 5 (sections 5.7 and 5.8). Herein, definition of variables specific to this chapter and statistical analyses are discussed.

7.2.1 DEFINITION OF VARIABLES

Reference age is defined as age at diagnosis for cases and age at participation (completion of questionnaire) for controls. Participants were asked to which ethnic groups they belonged, and in which country they were born; choices were given using the 2013 census categories. Participants could select more than one ethnicity. Participants who identified themselves with more than one ethnic group were assigned to a single ethnic group using prioritised ethnicity in the order of Māori, Pacific, and others (non-Pacific non-Māori). Country of birth was categorised into born and not born in New Zealand. Level of education was classified by the

highest qualification attained using the 2013 census categories. Income was based on the total personal pre-tax income in the last 12 months.

Ever-use and specifics of use of oral contraceptives (OCs), contraceptive implants, and 'other' contraceptives were queried. Participants were also asked whether they had ever used OCs for any purpose other than as a contraceptive. The relationship between use of contraceptive implants and the risk of ovarian cancer was not assessed because only 2 (1.3%) cases and 7 (0.9%) controls had ever-used contraceptive implants. The relationship between use of other contraceptives and the risk of ovarian cancer was also not assessed because this was a mix of contraceptive methods including natural, diaphragm, spermicides and cervical cap with different mechanisms of action. Participants were also asked whether they had a tubal ligation (TL) and if they had had a reversal operation. Only one control had had a reversal with the delivery of one child post-reversal and, therefore, the effect of reversal on the risk of ovarian cancer could not be assessed.

Information regarding ever-use of post-menopausal hormones (PMH), ovulation-inducing drugs, and condoms and their duration of use was sought. Parity included pre-term deliveries, term deliveries, and stillbirths. Women with one term delivery were assigned the same age for first and last delivery. Duration of breastfeeding was defined as total months of breastfeeding.

Any history of cancer included cancer of any site, either personal or in any blood relative (not limited to first-degree relatives); familial predisposition included any personal history of breast, endometrial or colorectal cancer, or history of breast, ovarian, endometrial, or colorectal cancer in a first-degree relative. Participants with a prior diagnosis of ovarian cancer were excluded from the study. Cumulative exposure to cigarettes in pack-years was calculated as number of years smoked multiplied by the average number of cigarettes smoked per day divided by 20. Height and weight were divided into quartiles based on the distribution in controls (the lowest quartile was used as the reference group). Weight in kilograms was divided by height in metres squared to obtain body mass index (BMI). BMI was classified into groups according to the WHO classification[4] [306].

[4] WHO classifies BMI as follows:
1. Underweight <18.50.
2. Normal range = 18.50-24.99.
3. Overweight ≥25.00 (Pre-obese = 25.00-29.99).
4. Obese ≥30.00: Obese class I = 30.00-34.99; Obese class II= 35.00-39.99; Obese class III ≥40.00.

7.2.2 DATA ANALYSIS

Data were analysed using the IBM Statistical Package for the Social Sciences (IBM SPSS statistics 22). All variables collected in the study were assessed for association with ovarian cancer, adjusted for age in 5-year groups using the method of Mantel and Haenszel [290]. When controlling for more than one variable, binary logistic regression was used. Odds ratios, 95% confidence intervals, and p-values were reported. Tests for trend were done using continuous rather than categorical variables.

Because duration of breastfeeding is correlated with parity, methods of controlling for parity were used [78]: the effect of breastfeeding was also assessed only in women who were parous (excluding nulliparous). In addition, age at first and last delivery and cumulative months of breastfeeding were adjusted for parity in a secondary analysis. For OCs, due to the possible influence of duration of use, age at first use of OCs and time since last use were also adjusted for duration of use.

The association between ovarian cancer and ever-use of condoms and between ovarian cancer and sterilisation was also assessed using women who had used neither hormonal contraceptives (defined as DMPA or OCs) nor the contraceptive being assessed as the reference group. Analyses restricted to women who had never used, and those who had ever-used, hormonal contraceptives were also done.

7.3 RESULTS

7.3.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS

The mean age of cases was 55.98 (SD = 8.04) and that of controls was 56.29 (SD = 9.00). This difference was not statistically significant (t = -0.414, df = 235, P = 0.679). There was also no difference in age distribution across the 7 five-year age-groups (χ^2 = 12.198, df = 6, P = 0.058). Compared to non-Pacific non-Māori women, being Māori was not associated with a higher risk of ovarian cancer (OR = 1.09; 95% CI = 0.58-2.06), nor was being Pacific (OR = 2.29; 95% CI = 0.93-5.64). No difference between cases and controls was observed in level of income, highest level of education attained, ethnicity, or country of birth. Results are presented in Table 7.1.

Table 7.1: Socio-demographic characteristics of study participants

	Cases		Controls		OR[1]	95% CI		P-value
	No.	*(%)*[2]	*No.*	*(%)*[2]		*Upper*	*Lower*	
Age								
35-39	5	(3)	43	(6)				
40-44	12	(8)	61	(8)				
45-49	18	(12)	91	(12)				
50-54	35	(23)	94	(13)				
55-59	29	(19)	156	(21)				
60-64	27	(18)	152	(20)				
65-69	26	(17)	149	(20)				
Country of Birth								
Not born in NZ	33	(22)	159	(21)	1.00			
Born in NZ	119	(78)	586	(78)	1.00	0.66	1.54	0.928
Ethnicity								
Non-Pacific non-Māori	132	(87)	670	(90)	1.00			
Māori	13	(9)	59	(8)	1.09	0.58	2.06	0.906
Pacific	7	(5)	17	(2)	2.29	0.93	5.64	0.114
Education								
No qualification	16	(11)	95	(13)	1.00			
Overseas Secondary school qualification	3	(2)	28	(4)	0.57	0.15	2.15	0.588
Level 1 or 2 Certificate	37	(25)	159	(21)	1.36	0.72	2.59	0.430
Level 3 or 4 Certificate	35	(24)	119	(16)	1.71	0.88	3.32	0.160
Level 5 or 6 Diploma	21	(14)	113	(15)	1.25	0.61	2.54	0.665
Bachelors Degree & Level 7 qualification	28	(19)	162	(22)	0.91	0.45	1.86	0.938
Postgraduate[3]	8	(5)	64	(9)	0.63	0.24	1.643	0.468
Trend Test – per category					*0.955*	*0.863*	*1.057*	*0.372*
Income								
≤$20,000 (Loss-$20,000)	34	(25)	186	(26)	1.00			
$20,001-$40,000	39	(29)	179	(25)	1.12	0.67	1.88	0.753
$40,001-$60,000	33	(24)	142	(20)	1.13	0.66	1.94	0.752
$60,001-$100,000	25	(18)	143	(20)	0.86	0.49	1.53	0.713
>$100,000 ($100,001 or More)	5	(4)	54	(8)	0.41	0.14	1.16	0.141
Trend Test – per category					*0.898*	*0.771*	*1.045*	*0.163*

[1]Adjusted for age in five-year groups

[2]Percentages are of total stated

[3]Postgraduate includes: postgraduate honours degree, Masters degree and Doctorate.

7.3.2 REPRODUCTIVE FACTORS

Age at menarche, cycle length, and menstrual regularity were not associated with ovarian cancer (Table 7.2). However, when compared with those ≤12 years of age at menarche, menarche at 14 years was associated with an OR of 1.45 (95% CI = 0.90-2.36). There was no association between age at sexual debut or lifetime number of sexual partners and ovarian cancer.

Overall, each year of later menopause was associated with an OR of 1.05 (95% CI = 1.01–1.08) (Table 7.2), which was due to higher risk in women who had surgical menopause: each year of later menopause was associated with an OR of 1.15 (95% CI = 1.08-1.23) in women with surgical menopause, whereas there was no association with natural menopause (OR = 1.00; 95% CI = 0.95-1.05).

A history of abortion (spontaneous or induced) was not associated with ovarian cancer (OR= 0.68; 95% CI = 0.46-1.01). Risk of ovarian cancer in relation to number of abortions was not assessed because the majority of women who had an abortion had only one (24/44 [55%] cases and 181/275 [66%] controls).

As shown in Table 7.2, parity was inversely associated with the risk of ovarian cancer, with a clear trend toward lower risk with higher parity (P-trend = 0.001). Compared to nulliparous women, women who had one term delivery had an OR of 0.79 (95% CI = 0.43-1.48); 2 deliveries (OR = 0.40, 95% CI = 0.24-0.67); 3 deliveries (OR = 0.53, 95% CI = 0.30-0.94) and ≥4 deliveries (OR = 0.33, 95% CI = 0.17–0.67).

First delivery at any age was inversely associated with risk, with delivery at >25 years of age attaining statistical significance (Table 7.3). However, no trend was observed (P-trend = 0.207). Compared to nulliparous women, last delivery at any age was inversely associated with ovarian cancer. The strength of this inverse association trended higher with later age at last delivery (P-trend = 0.001).

Compared to women who had never breastfed (parous only), a history of breastfeeding was associated with a statistically significant lower risk of ovarian cancer (OR= 0.49, 95% CI= 0.27-0.92). Each month of breastfeeding was associated with a 1.15% lower risk of ovarian cancer (OR= 0.99; 95% CI = 0.97-1.00). When the analysis included nulliparous women, no material change in the odd ratios was observed, and the inverse association between duration of breastfeeding and risk of ovarian cancer remained (P-trend = 0.001).

In a further attempt to disentangle the association with parity from that with breastfeeding and ages at first or last delivery, ORs were adjusted for parity (Table 7.3). When adjusted for parity, an inverse association across all ages at first delivery was observed, with the strength of association trending across later ages at first delivery (P-trend = 0.028). No change in the risk estimates associated with age at last delivery was noted after adjustment for parity. The inverse association with breastfeeding was slightly attenuated, but a statistically significant association with duration of breastfeeding remained (P-trend = 0.040).

Table 7.2: Association between reproductive factors and the risk of ovarian cancer

	Cases		Controls		OR[1]	95% CI		P-value
	No.	*(%)*[2]	*No.*	*(%)*[2]		*Lower*	*Upper*	
Age at Menarche								
≤ 12 (8.5-12.49)	53	(36)	280	(38)	1.00			
13 (12.50-13.49)	41	(28)	223	(30)	0.99	0.63	1.54	0.957
14 (13.50-14.49)	33	(22)	121	(17)	1.45	0.90	2.36	0.165
>14 (14.50-23.49)	22	(15)	109	(15)	1.04	0.60	1.81	0.998
Trend Test – per year					*1.014*	*0.902*	*1.139*	*0.819*
Age at Sexual Debut								
<16 (8-15)	16	(11)	62	(9)	1.00			
16-20	90	(63)	490	(70)	0.70	0.38	1.29	0.331
21-25	26	(18)	112	(16)	0.89	0.42	1.85	0.893
>25 (26-40)	11	(8)	33	(5)	1.33	0.50	3.54	0.748
Trend Test – per year					*1.040*	*0.989*	*1.093*	*0.124*
Number of partners								
0-1	42	(30)	204	(29)	1.00			
2-3	39	(28)	179	(25)	1.02	0.63	1.65	0.969
4-5	20	(14)	113	(16)	0.83	0.47	1.47	0.613
6-7	12	(9)	69	(10)	0.65	0.30	1.37	0.333
>7 (8-100)	28	(20)	144	(20)	0.85	0.48	1.50	0.676
Trend Test – per partner					*1.004*	*0.983*	*1.027*	*0.692*
Period Ceased								
No	46	(30)	229	(31)	1.00			
Yes	106	(70)	515	(69)	1.16	0.67	2.01	0.700
Age at Menopause								
≤45 (18-45)	24	(23)	144	(28)	1.00			
46-50	41	(39)	164	(31)	1.52	0.86	2.69	0.193
51-55	40	(38)	215	(41)	1.24	0.69	2.23	0.573
Trend Test – per year					*1.046*	*1.009*	*1.084*	*0.014*
Menopause Type								
Natural	82	(78)	421	(79)	1.00			
Surgical	23	(22)	110	(21)	1.03	0.61	1.73	0.987
Menstrual Regularity								
Regular	124	(82)	607	(82)	1.00			
Irregular	27	(18)	133	(18)	0.93	0.58	1.48	0.845
Cycle Length								
≤25 (18-25)	36	(28)	182	(27)	1.01	0.65	1.55	0.930
26-30	79	(61)	417	(63)	1.00			
31-35	10	(8)	47	(7)	1.30	0.62	2.72	0.632
>35 (36-90)	5	(4)	16	(2)	1.55	0.54	4.43	0.602
Trend Test – per day					*0.997*	*0.969*	*1.026*	*0.846*
Parity								
Nulliparous	32	(21)	85	(11)	1.00			
1	23	(15)	74	(10)	0.79	0.43	1.48	0.568
2	44	(29)	276	(37)	0.40	0.24	0.67	0.001
3	36	(24)	188	(25)	0.53	0.30	0.94	0.038
≥ 4 (4-7)	16	(11)	122	(16)	0.33	0.17	0.67	0.003
Trend Test – per birth					*0.794*	*0.690*	*0.913*	*0.001*
Abortions								
No	107	(71)	471	(63)	1.00			
Yes	44	(29)	274	(37)	0.68	0.46	1.00	0.065

[1]Adjusted for age in five-year groups

[2]Percentages are of total stated

Table 7.3: Risk of ovarian cancer in relation to parity, breastfeeding, and ages at first and last delivery

	Cases		Controls		OR[1]	95% CI		P-value	OR[2]	95% CI		P-value
	No.	*(%)*[3]	*No.*	*(%)*[3]		*Lower*	*Upper*			*Lower*	*Upper*	
Age at first delivery												
Nulliparous	32	(21)	85	(12)	1.00				1.00			
≤ 20 (15-20)	20	(13)	109	(15)	0.51	0.27	0.98	0.062	0.36	0.17	0.78	0.009
21-25	46	(30)	216	(29)	0.61	0.36	1.05	0.094	0.39	0.19	0.80	0.010
26-30	35	(23)	188	(25)	0.45	0.25	0.78	0.007	0.29	0.13	0.62	0.002
>30 (31-51)	18	(12)	141	(19)	0.35	0.18	0.66	0.002	0.17	0.07	0.41	<0.001
Trend Test - per year					*0.976*	*0.939*	*1.014*	*0.207*	*0.955*	*0.917*	*0.995*	*0.028*
Age at last delivery												
Nulliparous	32	(21)	85	(12)	1.00				1.00			
≤25 (18-25)	26	(17)	98	(13)	0.80	0.43	1.51	0.601	0.57	0.25	1.33	0.194
26-30	45	(30)	199	(27)	0.60	0.35	1.03	0.086	0.50	0.24	1.04	0.064
31-35	29	(19)	216	(29)	0.34	0.19	0.60	<0.001	0.28	0.13	0.59	0.001
>35 (36-53)	19	(13)	141	(19)	0.35	0.19	0.66	0.001	0.27	0.13	0.59	0.001
Trend Test – per year					*0.937*	*0.902*	*0.974*	*0.001*	*0.941*	*0.905*	*0.979*	*0.002*
Ever & Never Breastfed-Parous												
Never (parous)	16	(13)	46	(7)	1.00							
Ever	103	(87)	609	(93)	0.49	0.27	0.92	0.035	0.57	0.30	1.09	0.089
Breastfeeding Months (Parous)												
Never Breastfed (Parous)	16	(13)	46	(7)	1.00				1.00			
1-6	25	(21)	130	(20)	0.58	0.28	1.20	0.191	0.63	0.30	1.31	0.216
7-12	17	(14)	98	(15)	0.45	0.20	1.02	0.089	0.54	0.24	1.19	0.125
13-24	36	(30)	176	(27)	0.70	0.35	1.40	0.407	0.68	0.33	1.40	0.293
25-36	13	(11)	91	(14)	0.36	0.15	0.89	0.046	0.43	0.18	1.05	0.063
>36 (37-168)	12	(10)	114	(17)	0.32	0.13	0.78	0.014	0.34	0.14	0.85	0.020
Trend Test – per month					*0.985*	*0.973*	*0.997*	*0.012*	*0.987*	*0.974*	*0.999*	*0.040*

[1]Adjusted for age in five-year groups

[2]Adjusted for age in five-year groups, and parity (1, 2, 3, and ≥4)

[3]Percentages are of total stated

7.3.2.1 Use of oral contraceptives and condoms

Age-adjusted odds ratios for OC use and condom use were calculated (Table 7.4). Ever-use of OCs was associated with a statistically significant lower risk of ovarian cancer (OR = 0.35, 95% CI = 0.22-0.55). Longer duration of use was associated with lower risk (P-trend <0.001). The strongest inverse association was observed in users of OCs for 16-20 years (OR= 0.19; 95% CI = 0.09-0.42). The association trended lower with longer time since last use (P-trend <0.001). Women who last used OCs within the last 5 years had an OR of 0.10 (95% CI = 0.02-0.44), whereas those who last used OCs 26-30 years ago had an OR of 0.57 (95% CI = 0.30-1.11). The inverse association with OCs was evident 35 years after discontinuation of use (OR = 0.43, 95% CI = 0.21-0.90). There was no relationship with age at first use (P-trend = 0.081).

Participants were also asked about use of OCs for purposes other than as a contraceptive method. Reasons for 'other use' included treatment of abnormal menstrual pattern, dysmenorrhoea, endometriosis, acne, and polycystic ovary syndrome. The association between 'other use' of OCs and the risk of ovarian cancer mirrored that of use as a contraceptive.

Age at first use and time since last use were adjusted for total duration of use of OCs (Table 7.5). The association with time since discontinuation of use was attenuated, whereas no association was observed with age at initiation of use.

Only 2 cases and 7 controls had used DMPA but never used OCs; when they were excluded from the reference group, no change in the odds ratios was observed (Table 7.6). The ORs for ever-use of OCs were also unchanged when the analysis was restricted to women who had never used DMPA (bottom panel of Table 7.6).

In an analysis confined to ever-users of DMPA, the strength of the inverse association seen in ever-users of OCs was less marked and was no longer statistically significant (OR = 0.54; 95% CI = 0.10-3.02). However, this was based on a small number of participants; 11 cases and 82 controls were ever-users of OCs, and only 2 cases and 7 controls were never-users (Table 7.7).

As shown in Table 7.4, compared to never-use, condom use was not associated with ovarian cancer (OR = 0.77; 95% CI = 0.54-1.11). There was no relationship with duration of use (P-trend = 0.117). When compared to women who had never used OCs or DMPA (Table 7.6),

an inverse association with condom use was found (OR = 0.35; 95% CI = 0.19-0.65). However, when the analysis was limited to those who had never used OCs or DMPA, no association was observed with ever-use (OR = 0.96; 95% CI = 0.41-2.25), or duration of use (bottom panel of Table 7.6).

Condom use was also assessed in ever-users of OCs or DMPA (Table 7.7). There was no change in the odds ratio (OR = 0.79; 95% CI = 0.53-1.18) and there was no relationship with duration of use.

Table 7.4: Risk of ovarian cancer associated with use of oral contraceptives or condoms compared to never-use

	Cases		Controls		OR[1]	95% CI		P-value
	No.	*(%)[2]*	*No.*	*(%)[2]*		*Upper*	*Lower*	
Oral Contraceptives								
Never	36	(24)	77	(10)	1.00			
Ever	116	(76)	669	(90)	0.35	0.22	0.55	<0.001
Age at 1st Use								
Never Used OCs	36	(24)	77	(10)	1.00			
≤15 (11-15)	13	(9)	40	(5)	0.53	0.24	1.18	0.168
16-20	65	(43)	436	(59)	0.31	0.19	0.50	<0.001
21-25	23	(15)	141	(19)	0.39	0.21	0.72	0.003
>25 (26-36)	15	(10)	47	(6)	0.68	0.34	1.36	0.348
Trend Test – per year					*1.051*	*0.994*	*1.104*	*0.081*
Years Since Last Use								
Never Used OCs	36	(24)	77	(11)	1.00			
≤5 (0-5)	3	(2)	39	(6)	0.10	0.02	0.44	0.002
6-10	6	(4)	52	(8)	0.19	0.07	0.53	0.002
11-15	6	(4)	67	(10)	0.16	0.06	0.42	<0.001
16-20	17	(11)	104	(15)	0.28	0.14	0.57	<0.001
21-25	17	(11)	75	(11)	0.40	0.20	0.81	0.016
26-30	27	(18)	83	(12)	0.57	0.30	1.11	0.139
31-35	18	(12)	80	(12)	0.46	0.22	1.00	0.063
>35 (36-50)	18	(12)	116	(17)	0.43	0.21	0.90	0.040
Trend Test – per year					*1.051*	*1.023*	*1.080*	*<0.001*
OCs Use as a Contraceptive-Years								
Never Used OCs	36	(24)	77	(10)	1.00			
<1 (0)	17	(11)	35	(5)	0.88	0.42	1.84	0.876
1-5	38	(26)	179	(24)	0.43	0.25	0.75	0.004
6-10	28	(19)	168	(23)	0.30	0.17	0.55	<0.001
11-15	11	(7)	96	(13)	0.23	0.11	0.50	<0.001
16-20	9	(6)	100	(14)	0.19	0.09	0.42	<0.001
>20 (21-44)	9	(6)	80	(11)	0.21	0.09	0.49	<0.001
Trend Test – per year					*1.001*	*1.001*	*1.002*	*<0.001*
OC Use Ever-Other								
Never Used OCs	36	(24)	77	(10)	1.00			
No	100	(66)	550	(74)	0.37	0.23	0.59	<0.001
Yes	16	(11)	117	(16)	0.24	0.12	0.49	<0.001
OC Other Years of Use								
Never Used OCs	36	(71)	77	(40)	1.00			
<1 (0)	6	(12)	21	(11)	0.49	0.16	1.50	0.341
1-5	6	(12)	58	(30)	0.21	0.08	0.55	0.001
>5 (6-44)	3	(6)	36	(19)	0.12	0.03	0.49	0.003
Trend Test – per year					*0.992*	*0.901*	*1.093*	*0.869*
All Use OCs years								
Never Used OCs	36	(24)	77	(11)	1.00			
<1 (0)	17	(12)	34	(5)	0.91	0.44	1.91	0.955
1-5	38	(26)	177	(24)	0.44	0.25	0.76	0.004
6-10	26	(18)	167	(23)	0.29	0.16	0.53	<0.001
11-15	13	(9)	97	(13)	0.25	0.12	0.52	<0.001
16-20	9	(6)	101	(14)	0.19	0.09	0.41	<0.001
>20 (21-44)	8	(5)	80	(11)	0.20	0.09	0.47	<0.001
Trend Test – per year					*0.944*	*0.916*	*0.972*	*<0.001*
Condoms								
Never	82	(54)	365	(49)	1.00			
Ever	70	(46)	380	(51)	0.77	0.54	1.11	0.183
Condoms-Years of Use								
Never Used Condoms	82	(55)	365	(50)	1.00			
<1 (0)	9	(6)	47	(6)	0.79	0.38	1.65	0.640
1-5	31	(21)	204	(28)	0.61	0.39	0.97	0.046
6-10	12	(8)	59	(8)	0.82	0.42	1.60	0.665
>10 (11-40)	14	(9)	61	(8)	0.86	0.45	1.63	0.754
Trend Test – per year					*1.024*	*0.994*	*1.055*	*0.117*

[1]Adjusted for age in five-year groups

[2]Percentages are of total stated

Table 7.5: Risk of ovarian cancer in relation to age at first use and time since last use of oral contraceptives adjusted for age in 5-year groups and duration of use of oral contraceptives

	Cases		Controls		OR[1]	95% CI		P-Value
	No.	*(%)*[2]	*No.*	*(%)*[2]		*Lower*	*Upper*	
Age at 1st Use								
Never Used OCs	36	(24)	77	(10)	1.00			
≤15 (11-15)	13	(9)	40	(5)	0.51	0.18	1.42	0.194
16-20	65	(43)	436	(59)	0.17	0.07	0.40	<0.001
21-25	23	(15)	141	(19)	0.17	0.06	0.45	<0.001
>25 (26-36)	15	(10)	47	(6)	0.27	0.09	0.77	0.015
Trend Test – per year					*1.008*	*0.952*	*1.067*	*0.786*
Years Since Last Use								
Never Used OCs	36	(24)	77	(11)	1.00			
≤5 (0-5)	3	(2)	39	(6)	0.17	0.04	0.68	0.013
6-10	6	(4)	52	(8)	0.19	0.06	0.61	0.005
11-15	6	(4)	67	(10)	0.19	0.06	0.59	0.004
16-20	17	(11)	104	(15)	0.29	0.10	0.79	0.016
21-25	17	(11)	75	(11)	0.48	0.17	1.39	0.177
26-30	27	(18)	83	(12)	0.79	0.27	2.31	0.672
31-35	18	(12)	80	(12)	0.60	0.19	1.91	0.384
>35 (36-50)	18	(12)	116	(17)	0.42	0.13	1.35	0.144
Trend Test – per year					*1.038*	*1.003*	*1.075*	*0.034*

[1]Adjusted for age in five-year groups, and duration of use of oral contraceptives
[2]Percentages are of total stated

Table 7.6: Risk of ovarian cancer associated with use of oral contraceptives or condoms compared to never-use of DMPA, oral contraceptives and/or condoms

	Cases	Controls	OR[1]	95% CI		P-Value
	No. (%)[2]	*No. (%)*[2]		*Lower*	*Upper*	
Oral Contraceptives						
Ever-Use						
Never	34 (22)	70 (9)	1.00			
Ever	116 (76)	668 (90)	0.34	0.21	0.53	<0.001
Age at 1st Use						
Never Used OCs or DMPA	34 (22)	70 (9)	1.00			
≤15 (11-15)	13 (9)	40 (5)	0.51	0.23	1.15	0.150
16-20	65 (43)	436 (59)	0.30	0.18	0.49	<0.001
21-25	23 (15)	141 (19)	0.37	0.20	0.69	0.002
>25 (26-36)	15 (10)	47 (6)	0.66	0.33	1.34	0.326
Years Since Last Use						
Never Used OCs or DMPA	34 (23)	70 (10)	1.00			
≤5 (0-5)	3 (2)	39 (6)	0.09	0.02	0.43	0.002
6-10	6 (4)	52 (8)	0.17	0.06	0.48	0.001
11-15	6 (4)	67 (10)	0.13	0.05	0.37	<0.001
16-20	17 (12)	104 (15)	0.27	0.14	0.56	<0.001
21-25	17 (12)	75 (11)	0.38	0.18	0.77	0.012
26-30	27 (18)	83 (12)	0.55	0.28	1.08	0.122
31-35	18 (12)	80 (12)	0.44	0.21	0.93	0.051
>35 (36-50)	18 (12)	116 (17)	0.42	0.20	0.88	0.032
All Use OCs years						
Never Used OCs or DMPA	34 (23)	70 (10)	1.00			
<1 (0)	17 (12)	34 (5)	0.86	0.40	1.84	0.847
1-5	38 (26)	177 (24)	0.42	0.24	0.73	0.003
6-10	26 (18)	167 (23)	0.27	0.15	0.51	<0.001
11-15	13 (9)	97 (13)	0.24	0.11	0.50	<0.001
16-20	9 (6)	101 (14)	0.19	0.09	0.42	<0.001
>20 (21-44)	8 (6)	80 (11)	0.19	0.08	0.45	<0.001
Condoms						
Condoms Ever-Use						
Never	20 (22)	41 (10)	1.00			
Ever	70 (78)	380 (90)	0.35	0.19	0.65	0.001
Condoms-Years of Use						
Never	20 (23)	41 (10)	1.00			
<1 (0)	9 (10)	47 (11)	0.42	0.18	0.98	0.058
1-5	31 (36)	204 (50)	0.26	0.13	0.52	<0.001
6-10	12 (14)	59 (14)	0.37	0.15	0.89	0.037
>10 (11-40)	14 (16)	61 (15)	0.43	0.18	0.99	0.061
OCs Ever-Use (Non-DMPA)						
Never	34 (24)	70 (11)				
Ever	105 (76)	586 (89)	0.35	0.22	0.55	<0.001
Condoms ever-use (Non-Hormonal)						
Never	20 (59)	41 (59)	1.00			
Ever	14 (41)	28 (41)	0.96	0.41	2.25	0.895

[1]Adjusted for age in five-year groups
[2]Percentages are of total stated

Table 7.7: Risk of ovarian cancer associated with use of oral contraceptives or condoms in ever-users of DMPA

	Cases *No. (%)*[2]	Controls *No. (%)*[2]	OR[1]	95% CI *Lower*	*Upper*	P-Value
Oral Contraceptives						
Ever-Use						
Never	2 (15)	7 (8)	1.00			
Ever	11 (85)	82 (92)	0.54	0.10	3.02	0.842
Condoms						
Condoms Ever-Use						
Never	62 (53)	324 (48)	1.00			
Ever	56 (47)	352 (52)	0.79	0.53	1.18	0.291
Condoms-Years of Use						
Never Used Condoms	62 (54)	324 (49)	1.00			
<1 (0)	9 (8)	46 (7)	0.96	0.45	2.06	0.922
1-5	27 (23)	196 (29)	0.66	0.40	1.08	0.126
6-10	9 (8)	49 (7)	0.90	0.42	1.95	0.941
>10 (11-40)	8 (7)	52 (8)	0.66	0.29	1.50	0.418
Trend Test – per year			*1.000*	*1.000*	*1.001*	*0.197*

[1]Adjusted for age in five-year groups
[2]Percentages are of total stated

7.3.2.2 Ovulatory years

To assess whether the inverse association with use of OCs, parity, breastfeeding, and timing of first and last delivery could be partly explained by ovulations avoided, association between total ovulatory cycles and the risk of ovarian cancer was assessed.

7.3.2.2.1 Total ovulatory years estimation

In estimating total years of ovulatory cycles, women with a history of irregular menstrual cycles [85] and/or surgical menopause were excluded from the analysis (54 cases and 202 controls). Those with irregular periods are excluded because these cycles may not be ovulatory [85]. In addition, irregular menstrual cycles may be a pointer to ovarian pathology, such as polycystic ovary syndrome, which may also act to increase the risk of ovarian cancer [84]. Those with surgical menopause are not included because age at biologic menopause is not known. Also excluded, are those who did not answer questions on cycle length, type of menopause, menstrual regularity, parity, duration of breast feeding, number of abortions if any, and duration of OC use (2 cases and 53 controls). These variables were needed for the estimation of ovulatory years. These left 96 cases and 491 controls available for this analysis.

Total years of menstruation were calculated by subtracting age at menarche from age at last period for post-menopausal women or reference age if pre-menopausal (age at diagnosis for case subjects and age at completion of questionnaire for control subjects) [80, 83-85]. Lifetime ovulatory years were calculated by subtracting from total years of menstruation, the total years of anovulation, which was determined by term pregnancies, abortions, duration of

OC use, and total duration of breastfeeding. Lifetime ovulatory cycles were then calculated by multiplying the lifetime ovulatory years by the estimated number of cycles per year based on a woman's stated cycle length (365/cycle length) [80, 83, 85]. Finally, total ovulatory years (total years of ovulatory cycles) were computed by dividing the lifetime ovulatory cycles by 13 (average cycles per year in a normal 28 days cycle =3 65/28 = 13, which is equivalent to 13 ovulations per year) [80, 82].

In calculating total anovulatory years, 9 anovulatory months was attributed to each term delivery, and 3 anovulatory months for every abortion [83]. Term pregnancy lasts 37-41 weeks, which is approximately 9 months, and about 80% of spontaneous abortions occur before 12 weeks of pregnancy [23]. Those who did not indicate their age at menopause (1 case and 2 controls) were assigned 50 years (the mean, median, and mode age at menopause for control women with natural menopause). Participants with missing values for age at menarche (0 cases and 7controls) were assigned 13 years (the mean, median, and mode age at menarche for control women) [85]. For duration of OC use, 6 months was assigned to those who had used OCs for less than one year.

7.3.2.2.2 Findings

The mean ovulatory years for cases was 28.8 (SD = 9.4) and controls was 23.0 (SD = 11.0), a difference that was statistically significant (t = 5.389; df = 151; P <0.001). Thus, case subjects had on average 5.8 ovulatory years more than the controls.

As presented in Table 7.8, each year of ovulation was associated with a 5% higher risk of ovarian cancer (OR = 1.05; 95% CI = 1.03–1.08). Compared to the first quartile, women with ovulatory cycles in the fourth quartile had the highest risk of ovarian cancer (OR = 4.45; 95% CI = 2.00-9.87).

After adjusting for ovulatory years, compared to age adjusted analysis (Tables 7.2 and 7.3), parity, age at first and last delivery, and breastfeeding (all and when restricted to parous women) were no longer associated with risk of ovarian cancer (Table 7.9). There was also no dose-response relationship for any of these variables except age at last delivery, where each year of later age at last birth was associated with a 5% lower risk of ovarian cancer (OR = 0.95; 95% CI = 0.90-0.99).

Compared to age-adjusted analysis (Table 7.4), the odds ratios associated with use of OCs were slightly attenuated (OR = 0.35 in age-adjusted versus OR = 0.42 in age- and ovulatory-

years-adjusted analysis for ever-use), but the association was still statistically significant (OR = 0.42; 95% CI = 0.23-0.78 for ever-use). The inverse association was attenuated with greater time since last use, and a trend toward lower risk with longer duration of use was observed (P-trend = 0.009, and 0.060, respectively). There was no association with age at initiation of use of OCs. Ever-use and duration of use of condoms were not associated with the risk of ovarian cancer (Table 7.10).

Table 7.8: Age-adjusted risk estimates of ovarian cancer in relation to years of ovulatory cycles

	Cases *No. (%)*[2]	**Controls** *No. (%)*[2]	**OR**[1]	**95% CI** *Lower Upper*	**P-Value**
Years of Ovulatory Cycles[3]					
Quartile 1(<15= 0.63-14.99)	10 (10)	126 (26)	1.00		
Quartile 2 (15.00-23.99)	12 (13)	125 (25)	1.21	0.49 2.98	0.851
Quartile 3 (24.00-30.49)	29 (30)	118 (24)	3.10	1.39 7.02	0.009
Quartile 4 (30.50-56.15)	45 (47)	122 (25)	4.45	2.00 9.87	<0.001
Trend test – per year			*1.05*	*1.03 1.08*	*<0.001*

[1]Percentages are of total stated
[2]Adjusted for age in five-year groups
[3]Ovulatory years were categorised into quartiles based on the distribution of controls.

Table 7.9: Relationship between ovarian cancer and parity, age at first and last delivery, breastfeeding (parous women), adjusted for age in 5-year groups and ovulatory years

	Cases		Controls		OR[1]	95% CI		P-value
	No.	*(%)*[2]	*No.*	*(%)*[2]		*Lower*	*Upper*	
Parity								
Nulliparous	15	(16)	64	(13)	1.00			
1	14	(15)	52	(11)	1.64	0.69	3.91	0.264
2	29	(30)	190	(39)	0.74	0.36	1.52	0.413
3	26	(27)	116	(24)	1.16	0.55	2.44	0.700
≥ 4 (4-7)	12	(13)	69	(14)	0.90	0.37	2.17	0.816
Trend Test – per birth					*0.99*	*0.83*	*1.18*	*0.923*
Age at first delivery								
Nulliparous	15	(16)	64	(13)	1.00			
≤ 20 (15-20)	12	(13)	52	(11)	1.21	0.50	2.94	0.673
21-25	32	(33)	136	(28)	1.16	0.56	2.38	0.696
26-30	24	(25)	128	(26)	0.89	0.42	1.86	0.746
>30 (31-51)	13	(14)	111	(23)	0.72	0.31	1.67	0.441
Trend Test – per year					*0.97*	*0.92*	*1.02*	*0.179*
Age at last delivery								
Nulliparous	15	(16)	64	(13)	1.00			
≤25 (18-25)	16	(17)	56	(11)	1.36	0.59	3.13	0.469
26-30	33	(34)	114	(23)	1.37	0.67	2.81	0.388
31-35	19	(20)	156	(32)	0.62	0.29	1.33	0.220
>35 (36-53)	13	(14)	101	(21)	0.76	0.33	1.78	0.528
Trend Test – per year					*0.95*	*0.90*	*0.99*	*0.028*
Ever & Never Breastfed-Parous								
Never (parous)	10	(12)	29	(7)	1.00			
Ever	71	(88)	398	(93)	0.61	0.27	1.36	0.228
Breastfeeding Months (Parous)								
Never (Parous)	10	(12)	29	(7)	1.00			
1-6	19	(23)	84	(20)	0.74	0.30	1.86	0.527
7-12	9	(11)	59	(14)	0.54	0.19	1.54	0.246
13-24	23	(28)	110	(26)	0.69	0.28	1.68	0.412
25-36	10	(12)	63	(15)	0.54	0.19	1.51	0.240
>36 (37-168)	10	(12)	82	(19)	0.43	0.15	1.19	0.102
Trend Test – per month					*0.99*	*0.98*	*1.00*	*0.140*

[1]Adjusted for age in five-year groups, and ovulatory years (grouped into quartiles)
[2]Percentages are of total stated

Table 7.10: Relationship between ovarian cancer and use of oral contraceptives or condoms adjusted for age in 5-year groups and ovulatory years

	Cases		Controls		OR[1]	95% CI		P-value
	No.	*(%)*[2]	*No.*	*(%)*[2]		*Lower*	*Upper*	
Oral contraceptives								
Ever-Use								
No	25	(26)	45	(9)	1.00			
Yes	71	(74)	446	(91)	0.42	0.23	0.78	0.005
Age 1st Use								
Never Used OCs	25	(26)	45	(9)	1.00			
≤15 (11-15)	7	(7)	26	(5)	0.85	0.29	2.51	0.772
16-20	41	(43)	292	(60)	0.40	0.20	0.77	0.006
21-25	13	(14)	94	(19)	0.34	0.15	0.75	0.007
>25 (26-36)	10	(10)	32	(7)	0.73	0.29	1.79	0.486
Trend Test – per year					*1.02*	*0.95*	*1.09*	*0.550*
Years Since Last Use								
Never Used OCs	25	(26)	45	(10)	1.00			
≤10 (0-10)	4	(4)	71	(16)	0.10	0.02	0.41	0.001
11-15	4	(4)	50	(11)	0.15	0.04	0.55	0.004
16-20	7	(7)	80	(17)	0.16	0.06	0.44	<0.001
21-25	14	(15)	53	(12)	0.51	0.22	1.18	0.115
26-30	17	(18)	49	(11)	0.76	0.34	1.68	0.492
31-35	15	(16)	49	(11)	0.72	0.31	1.63	0.424
>35 (36-50)	9	(9)	61	(13)	0.39	0.16	0.95	0.039
Trend Test – per year					*1.06*	*1.01*	*1.11*	*0.009*
All Use OCs years								
Never Used OCs	25	(26)	45	(9)	1.00			
<1 (0)	10	(10)	25	(5)	0.72	0.29	1.81	0.487
1-5	23	(24)	112	(23)	0.40	0.20	0.79	0.008
6-10	23	(24)	107	(22)	0.44	0.21	0.91	0.028
11-15	8	(8)	71	(14)	0.25	0.09	0.68	0.007
16-20	3	(3)	77	(16)	0.06	0.01	0.29	<0.001
>20 (21-44)	4	(4)	54	(11)	0.11	0.02	0.54	0.006
Trend Test – per year					*0.94*	*0.89*	*1.00*	*0.060*
Condoms								
Condoms Ever-Use								
Never	46	(48)	228	(47)	1.00			
Ever	50	(52)	262	(53)	0.91	0.56	1.46	.684
Condoms-Years of Use								
Never Used Condoms	46	(48)	228	(47)	1.00			
<1 (0)	6	(6)	28	(6)	0.95	0.37	2.58	0.954
1-5	23	(24)	135	(28)	0.90	0.51	1.60	0.731
6-10	10	(11)	41	(8)	1.10	0.49	2.52	0.806
>10 (11-40)	10	(11)	54	(11)	0.73	0.33	1.61	0.429
Trend Test – per year					*1.00*	*0.97*	*1.04*	*0.852*

[1]Adjusted for age in five-year groups, and ovulatory years (grouped into quartiles)
[2]Percentages are of total stated

7.3.3 GYNAECOLOGICAL OPERATIONS

Compared to women with intact ovaries, no association was observed between ovarian cancer and a history of unilateral oophorectomy (OR = 0.63; 95% CI = 0.24-1.67). The numbers were too small to assess for trend: 5 cases and 35 controls had undergone unilateral oophorectomy. However, when women above and below the controls' median age at

oophorectomy were compared with women with both ovaries intact, there was no difference in risk between the two groups (Table 7.11).

Compared to women with an intact uterus, history of hysterectomy was not associated with ovarian cancer (OR = 0.71; 95% CI = 0.41-1.23). No relationship between risk and age at hysterectomy was observed (P-trend = 0.402).

History of tubal ligation (TL) was not associated with the risk of ovarian cancer (OR = 0.98; 95% CI = 0.62-1.57). There was also no relationship with age at TL or time since tubal ligation. However, compared to unsterilized women who had never used hormonal contraceptives (DMPA or OCs), a statistically significant lower risk of ovarian cancer was observed in women with history of TL (OR = 0.38; 95% CI = 0.20-0.72). There was no relationship between risk and the age at TL or time since operation (Table 7.12).

In an analysis confined to women who had never used hormonal contraceptives, no association was observed in sterilised compared to unsterilised women (OR = 0.61; 95% CI = 0.16-2.39) (Table 7.12, bottom panel). A small number of women (3 cases and 12 controls) had a history of tubal ligation and had never used hormonal contraceptives.

Among ever-users of hormonal contraceptives, history of TL was not associated with the risk of ovarian cancer (OR = 1.14; 95% CI = 0.69-1.90). No relationship with age at, or time since, TL was observed (Table 7.13). In addition, no association was observed between TL and risk of ovarian cancer when the analysis was adjusted for ovulatory years (Table 7.14).

Table 7.11: Risk of ovarian cancer in women with history of unilateral oophorectomy, hysterectomy, and tubal ligation

	Cases	Controls	OR[1]	95% CI		P-value
	No. (%)[2]	*No. (%)*[2]		*Upper*	*Lower*	
Unilateral Oophorectomy						
No	147 (97)	711 (95)	1.00			
Yes	5 (3)	35 (5)	0.63	0.24	1.67	0.473
Unilateral Oophorectomy - Age						
Not Had Oophorectomy	147 (97)	711 (95)	1.00			
≤36 (15-36)	2 (1)	18 (2)	0.50	0.11	2.24	0.538
>36 (37-63)	3 (2)	17 (2)	0.77	0.22	2.69	0.912
Hysterectomy						
No	134 (88)	630 (84)	1.00			
Yes	18 (12)	116 (16)	0.71	0.41	1.23	0.275
Hysterectomy - Age						
Not had hysterectomy	134 (88)	630 (84)	1.00			
≤35 (27-35)	3 (2)	27 (4)	0.55	0.16	1.85	0.467
36-40	4 (3)	27 (4)	0.72	0.24	2.04	0.680
41-45	4 (3)	25 (3)	0.70	0.23	2.11	0.702
46-50	5 (3)	20 (3)	1.12	0.41	3.05	0.967
>50 (51-64)	2 (1)	17 (2)	0.55	0.13	2.38	0.605
Trend Test – per year			1.028	0.964	1.095	0.402
Tubal Ligation						
No	121 (82)	600 (80)	1.00			
Yes	27 (18)	146 (20)	0.98	0.62	1.57	0.963
Age at TL						
Never had TL	121 (82)	600 (80)	1.00			
≤30 (22-30)	10 (7)	57 (8)	0.94	0.46	1.92	0.999
31-35	8 (5)	46 (6)	0.92	0.42	2.02	0.997
36-40	7 (5)	26 (3)	1.37	0.58	3.28	0.638
>40 (41-56)	2 (1)	17 (2)	0.581	0.13	2.54	0.673
Trend Test- per year			1.001	0.929	1.079	0.980
Years Since TL						
Never had TL	121 (82)	600 (80)	1.00			
≤10 (1-10)	2 (1)	10 (1)	1.01	0.21	4.76	0.697
11-20	6 (4)	18 (2)	1.40	0.53	3.66	0.675
21-30	9 (6)	54 (7)	0.88	0.42	1.86	0.877
>30 (31-45)	10 (7)	64 (9)	0.91	0.44	1.91	0.953
Trend Test – per year			*0.994*	*0.925*	*1.067*	*0.860*

[1]Adjusted for age in five-year groups
[2]Percentages are of total stated

Table 7.12: Risk of ovarian cancer in sterilised women compared to unsterilized women who had never used oral contraceptives or DMPA

	Cases *No. (%)*[2]	Controls *No. (%)*[2]	OR[1]	95% CI *Lower Upper*	P-Value
Ever had TL					
No (None)	30 (53)	57 (28)	1.00		
Yes	27 (47)	146 (72)	0.38	0.20 0.72	0.004
Age TL					
Never had TL or Used DMPA/OCs	30 (53)	57 (28)	1.00		
≤30 (22-30)	10 (18)	57 (28)	0.35	0.15 0.83	0.028
31-35	8 (14)	46 (23)	0.36	0.14 0.89	0.042
36-40	7 (12)	26 (13)	0.54	0.20 1.40	0.294
>40 (41-56)	2 (4)	17 (8)	0.24	0.05 1.10	0.091
Years Since TL					
Never had TL or Used DMPA/OCs	30 (53)	57 (28)	1.00		
≤10 (1-10)	2 (4)	10 (5)	0.28	0.05 1.72	0.296
11-20	6 (11)	18 (9)	0.51	0.16 1.60	0.380
21-30	9 (16)	54 (27)	0.37	0.15 0.90	0.044
>30 (31-45)	10 (18)	64 (32)	0.35	0.14 0.91	0.048
Never-used hormonal contraceptives					
Tubal Ligation (Non-Hormonal)					
No	30 (91)	57 (83)	1.00		
Yes	3 (9)	12 (17)	0.61	0.16 2.40	0.700

[1]Adjusted for age in five-year groups
[2]Percentages are of total stated

Table 7.13: Association between tubal ligation and the risk of ovarian cancer in ever-users of DMPA and/or oral contraceptives

	Cases *No. (%)*[2]	Controls *No. (%)*[2]	OR[1]	95% CI *Lower Upper*	P-Value
Ever had TL					
No TL; Yes OCs/DMPA	91 (79)	543 (80)	1.00		
Yes TL & Yes OCs/DMPA	24 (21)	134 (20)	1.14	0.69 1.90	0.705
Age TL					
Never had TL	91 (79)	543 (80)	1.00		
≤30 (22-30)	9 (8)	50 (7)	1.17	0.55 2.52	0.836
31-35	7 (6)	45 (7)	1.00	0.43 2.32	0.835
36-40	6 (5)	24 (4)	1.56	0.61 3.97	0.515
>40 (41-56)	2 (2)	15 (2)	0.79	0.18 3.48	0.973
Trend Test – per year			*0.998*	*0.922 1.080*	*0.953*
Years Since TL					
Never had TL	91 (79)	543 (80)	1.00		
≤10 (1-10)	1 (1)	9 (1)	0.68	0.08 5.51	0.921
11-20	5 (4)	16 (2)	1.46	0.51 4.17	0.674
21-30	9 (8)	52 (8)	1.09	0.51 2.33	0.982
>30 (31-45)	9 (8)	57 (8)	1.18	0.53 2.63	0.843
Trend Test – per year			*0.995*	*0.921 1.074*	*0.890*

[1]Adjusted for age in five-year groups
[2]Percentages are of total stated

Table 7.14: Risk of ovarian cancer in sterilised women compared to unsterilized women, adjusted for age and ovulatory years

	Cases	Controls	OR[1]	95% CI	P-value
	No. (%)[2]	*No. (%)*[2]		*Lower Upper*	
Ever had TL					
No	80 (82)	(83) 412	1.00		
Yes	16 (18)	(17) 79	0.97	0.52 1.82	0.925
Age TL					
Never had TL	80 (83)	412 (84)	1.00		
≤30 (22-30)	7 (7)	32 (7)	0.91	0.37 2.21	0.825
31-35	6 (6)	22 (4)	1.53	0.57 4.09	0.397
36-40	2 (2)	18 (4)	0.60	0.13 2.74	0.509
>40 (41-56)	1 (1)	7 (1)	0.69	0.08 6.13	0.739
Trend Test – per year			*0.968*	*0.851 1.102*	*0.627*
Years since TL					
Never had TL	80 (83)	412 (84)	1.00		
≤20 (1-20)	4 (4)	18 (4)	1.15	0.36 3.73	0.811
21-30	6 (6)	28 (6)	1.19	0.46 3.09	0.725
>30 (31-45)	6 (6)	33 (7)	0.72	0.27 1.93	0.511
Trend Test – per year			*1.034*	*0.915 1.168*	*0.589*

[1]Adjusted for age in five-year groups, and ovulatory years in quartiles
[2]Percentages are of total stated

7.3.4 HISTORY OF GYNAECOLOGICAL CONDITIONS AND CANCER

Endometriosis and infertility were positively associated with ovarian cancer (OR = 2.75; 95% CI = 1.62-4.68, and OR = 3.05; 95% CI = 1.52-6.12, respectively), whereas fibroids and benign ovarian cysts were not associated (Table 7.15). The association with infertility was not altered by adjusting for ever-use of ovulation-inducing drugs (OR = 3.65; 95% CI = 1.47-9.05) (data not shown).

A family history of cancer at any site in any relative was associated with a statistically significantly higher risk of ovarian cancer (OR = 2.00; 95% CI = 1.26-3.18). When analysis was restricted to women with a history of breast, ovarian, endometrial, or colorectal cancer in a first-degree relative, the confidence limits just included 1.0: OR = 1.43; 95% CI = 0.98-2.08.

Table 7.15: Relationship between gynaecological conditions and a family or personal history of cancer with the risk of ovarian cancer

	Case *No. (%)*[2]	Controls *No. (%)*[2]	OR[1]	95% CI *Upper Lower*	P-value
Fibroids					
No	126 (84)	632 (86)	1.00		
Yes	24 (16)	106 (14)	1.07	0.65 1.74	0.898
Endometriosis					
No	126 (84)	687 (94)	1.00		
Yes	24 (16)	46 (6)	2.75	1.62 4.68	<0.001
Benign Ovarian Cysts					
No	132 (89)	671 (91)	1.00		
Yes	17 (11)	64 (9)	1.257	0.711 2.221	0.523
Infertility					
No	135 (91)	704 (96)	1.00		
Yes	14 (9)	26 (4)	3.05	1.52 6.12	0.002
Familial Predisposition[3]					
No	95 (63)	519 (70)	1.00		
Yes	55 (37)	218 (30)	1.43	0.98 2.08	0.080
Any Cancer History[4]					
No	26 (17)	212 (29)	1.00		
Yes	125 (83)	531 (71)	2.00	1.26 3.18	0.004

[1]Adjusted for age in five-year groups

[2]Percentages are of total stated

[3]Personal history and/or history of breast, ovarian, endometrial or colorectal cancer in a first-degree relative

[4]Personal history and/or history of cancer of any site in any relative

7.3.5 USE OF PMH AND OVULATION-INDUCING DRUGS

As shown in Table 7.16, no association with ovarian cancer was observed in ever-users of ovulation-inducing drugs compared to never-users (OR = 1.55; 95% CI = 0.78-3.05). There was a suggestion of lower risk with longer duration of use: compared to never-use, use for 0-3, 4-6, and >6 months were associated with ORs of 2.20, 1.67, and 0.64, respectively, although the p-value for trend was not statistically significant (P-trend = 0.140). Use of ovulation-inducing drugs was not associated with ovarian cancer after adjustment for infertility (OR = 0.75; 95% CI = 0.30-1.88) (data not shown).

A statistically significant positive association with ovarian cancer was observed in ever-users compared to never-users of PMH (OR = 1.74, 95% CI = 1.09-2.75). No duration-response relationship was observed (P-trend = 0.148).

Table 7.16: Risk of ovarian cancer associated with use of PMH and ovulation-inducing drugs

	Cases	Controls	OR[1]	95% CI		P-value
	No. (%)[2]	*No. (%)*[2]		*Upper*	*Lower*	
Use of fertility drugs						
No	139 (92)	706 (95)	1.00			
Yes	12 (8)	39 (5)	1.55	0.78	3.05	0.286
Fertility drugs use - Months						
Never Used fertility Drugs	139 (93)	706 (95)	1.00			
0-3	7 (5)	15 (2)	2.20	0.86	5.65	0.170
4-6	2 (1)	6 (1)	1.67	0.34	8.23	0.865
>6 (7-60)	2 (1)	17 (2)	0.64	0.15	2.76	0.768
Trend Test – per month			*0.879*	*0.741*	*1.043*	*0.140*
PMH Use						
No	117 (77)	627 (84)	1.00			
Yes	34 (23)	117 (16)	1.74	1.09	2.75	0.025
PMH Use - Years						
Never Used PMH	117 (77)	627 (84)	1.00			
0-2.49 (0-2)	19 (13)	43 (6)	2.44	1.35	4.41	0.004
2.50-4.49 (3-4)	4 (3)	23 (3)	1.08	0.35	3.27	0.874
4.50-6.49 (5-6)	6 (4)	13 (2)	2.78	1.01	7.68	0.087
6.50-33.49 (≥7[7-33])	5 (3)	38 (5)	0.82	0.30	2.21	0.876
Trend Test – per year			*0.942*	*0.868*	*1.022*	*0.148*

[1]Adjusted for age in five-year groups
[2]Percentages are of total stated

7.3.6 LIFESTYLE FACTORS

As shown in Table 7.17, compared to never-use, a statistically significant positive association was observed between ever-use of talcum powder in the perineal area and ovarian cancer (OR = 1.54; 95% CI = 1.05-2.25). There was no relationship with duration of use (P-trend = 0.839).

Relative to non-drinkers, alcohol consumption within the last ten years was not associated with ovarian cancer (OR = 0.67; 95% CI = 0.44-1.03).

History of smoking was not associated with ovarian cancer (OR = 0.95; 95% CI = 0.66-1.36). There was no difference in risk between current and past smokers. A linear relationship was observed between duration of smoking and ovarian cancer (OR = 1.02; 95% CI = 1.00-1.04 for each year of smoking, P-trend = 0.049). A clear trend toward lower risk with time since quitting smoking was also observed (P-trend = 0.001). No association was observed with number of pack-years (P-trend = 0.125).

Table 7.17: Risk of ovarian cancer in relation to talcum use, smoking, and alcohol use

	Cases		Controls		OR[1]	95% CI		P-value
	No.	*(%)*[2]	*No.*	*(%)*[2]		*Upper*	*Lower*	
Talcum Use								
No	97	(65)	546	(74)	1.00			
Yes	52	(35)	194	(26)	1.54	1.05	2.25	0.034
Talcum Use Years								
Never Used Talcum	97	(66)	546	(75)	1.00			
<1 (0)	6	(4)	46	(6)	0.65	0.27	1.57	0.444
1-10	22	(15)	60	(8)	2.10	1.22	3.63	0.010
11-20	11	(8)	20	(3)	3.14	1.45	6.79	0.004
>20 (21-69)	10	(7)	52	(7)	1.21	0.59	2.51	0.741
Trend Test – per year					*1.00*	*0.98*	*1.02*	*0.839*
Alcohol Use								
No	35	(23)	126	(17)	1.00			
Yes	116	(77)	618	(83)	0.67	0.44	1.03	0.087
Smoking								
Never	91	(60)	443	(59)	1.00			
Ever	60	(40)	303	(41)	0.95	0.66	1.36	0.854
Smoking Status								
Never Smoker	91	(60)	443	(59)	1.00			
Ex-Smoker (Past Smoker)	48	(32)	236	(32)	0.98	0.67	1.45	0.987
Current Smoker	12	(8)	67	(9)	0.86	0.44	1.67	0.775
Years Smoked								
Never Smoker	91	(60)	443	(60)	1.00			
0-10	16	(11)	85	(11)	0.89	0.50	1.60	0.814
11-20	9	(6)	66	(9)	0.68	0.33	1.41	0.380
21-30	9	(6)	65	(9)	0.65	0.31	1.39	0.350
>30 (31-52)	26	(17)	82	(11)	1.57	0.94	2.60	0.109
Trend Test – per year					*1.02*	*1.00*	*1.04*	*0.049*
Pack Years								
Never smoker	91	(60)	443	(60)	1.00			
≤1 (0.00-1.00)	3	(2)	32	(4)	0.43	0.12	1.49	0.272
1.01-5.00	16	(11)	69	(9)	1.14	0.63	2.08	0.790
5.01-10.00	13	(9)	40	(5)	1.49	0.75	2.93	0.340
10.01-20.00	10	(7)	79	(11)	0.61	0.31	1.23	0.214
>20.00 (20.01-78.00)	18	(12)	76	(10)	1.14	0.65	2.02	0.758
Trend Test – per year					*1.02*	*1.00*	*1.04*	*0.125*
Years Last smoked								
Never Smoker	91	(60)	443	(60)	1.00			
Current Smoker	12	(8)	67	(9)	0.86	0.44	1.67	0.775
0-5	18	(12)	27	(4)	3.60	1.86	6.99	<0.001
6-10	5	(3)	25	(3)	1.10	0.40	3.02	0.931
11-20	7	(5)	44	(6)	0.77	0.33	1.79	0.689
21-30	7	(5)	68	(9)	0.45	0.20	1.03	0.076
>30 (31-49)	11	(7)	65	(9)	0.81	0.41	1.62	0.669
Trend Test – per year					0.95	0.93	0.98	0.001

[1]Adjusted for age in five-year groups
[2]Percentages are of total stated

7.3.7 ANTHROPOMETRIC MEASURES

As presented in Table 7.18, current weight was inversely associated with ovarian cancer: compared to those in the lowest quartile, women in the highest quartile had an OR of 0.60 (95% CI = 0.36-1.00). A statistically significant trend toward lower risk with greater weight was observed (P-trend = 0.029). Similar to current weight, higher current BMI was inversely associated with ovarian cancer. Compared to BMI within the normal range (18.50-24.99 kg/m^2), being obese (BMI $\geq$30 kg/m^2) was associated with an OR of 0.60 (95% CI = 0.38-0.97); a statistically significant trend was observed (P-trend = 0.020). In contrast, there was no association with usual adult weight or BMI. Compared to women with usual weight in the first quartile, women with usual weight in the second, third, and fourth quartiles had ORs of 1.03, 1.20, and 1.31, respectively, although the p-value for trend was not statistically significant (P-trend = 0.195). Compared to usual BMI within the normal range, a usual BMI of 25.00-29.99 kg/m^2 and $\geq$30 kg/m^2 were associated with an OR of 0.93 (95% CI = 0.59-1.45) and 1.56 (95% CI = 0.95-2.58), respectively, whereas a usual BMI of <18.50 kg/m^2 was associated with an OR of 1.77 (95% CI = 0.72-4.39). There was no association between ovarian cancer and height (P-trend = 0.936), weight, or BMI at 18 years (P-trend = 0.805 and 0.729, respectively).

Table 7.18: Risk of ovarian cancer in relation to height, weight, and BMI at different stages of life

	Cases		Controls		OR[1]	95% CI		P-value
	No.	*(%)*[2]	*No.*	*(%)*[2]		*Upper*	*Lower*	
Height (cm)								
≤160 (132-160)	56	(37)	247	(33)	1.00			
161-164	27	(18)	133	(18)	0.86	0.52	1.44	0.664
165-170	40	(27)	236	(32)	0.72	0.46	1.13	0.184
>170 (171-206)	27	(18)	122	(17)	0.94	0.56	1.58	0.912
Trend Test – per cm					*1.001*	*0.977*	*1.025*	*0.936*
Current Weight (Kgs)								
≤ 62 (42-62)	53	(35)	199	(27)	1.00			
63-70	38	(25)	174	(24)	0.84	0.52	1.33	0.524
71-82	33	(22)	193	(26)	0.64	0.40	1.02	0.076
>82 (83-164)	26	(17)	169	(23)	0.60	0.36	1.00	0.062
Trend Test – per Kg					*0.987*	*0.976*	*0.999*	*0.029*
Current BMI (Kg/M^2)								
<18.50 (16.94-18.49)	5	(3)	13	(2)	1.41	0.46	4.30	0.770
18.50-24.99	75	(50)	289	(40)	1.00			
25.00-29.99	40	(27)	225	(31)	0.69	0.45	1.05	0.102
≥30 (30.00-67.21)	30	(20)	201	(28)	0.60	0.38	0.96	0.040
Trend test – per Kg/M^2					*0.961*	*0.930*	*0.994*	*0.020*
Weight at 18 Years (Kgs)								
30-52	35	(27)	181	(26)	1.00			
53-59	33	(25)	170	(25)	0.98	0.57	1.66	0.969
60-65	32	(24)	187	(27)	0.85	0.50	1.45	0.649
> 65 (66-178)	31	(24)	152	(22)	1.10	0.65	1.89	0.824
Trend Test – per Kg					*1.002*	*0.987*	*1.018*	*0.805*
BMI at 18 Years (Kg/M^2)								
< 18.50 (11.29-18.49)	20	(15)	83	(12)	1.39	0.80	2.42	0.312
18.50-24.99	87	(66)	494	(72)	1.00			
25.00-29.99	17	(13)	81	(12)	1.27	0.71	2.26	0.526
≥30 (30.00-63.07)	7	(5)	26	(4)	1.66	0.70	3.98	0.369
Trend Test – per Kg/M^2					*1.008*	*0.964*	*1.054*	*0.729*
Usual Weight								
38-57	33	(23)	182	(26)	1.00			
58-65	40	(28)	212	(30)	1.03	0.62	1.71	0.984
66-75	39	(27)	173	(25)	1.20	0.71	2.01	0.587
>75 (76-178)	33	(23)	139	(20)	1.31	0.76	2.23	0.401
Trend Test – per Kg					*1.008*	*0.996*	*1.021*	*0.195*
Usual BMI (Kg/M^2)								
<18.50 (14.53-18.49)	7	(5)	21	(3)	1.77	0.72	4.39	0.320
18.50-24.99	78	(54)	402	(57)	1.00			
25.00-29.99	34	(23)	190	(27)	0.928	0.59	1.45	0.829
≥30 (30.00-63.07)	26	(18)	87	(12)	1.560	0.95	2.58	0.107
Trend test – per Kg/M^2					*1.026*	*0.990*	*1.062*	*0.160*

[1]Adjusted for age in five-year groups
[2]Percentages are of total stated

7.4 DISCUSSION

A detailed discussion of previous studies that investigated the association between ovarian cancer and factors that may modify risk and their possible biologic mechanisms of association have been provided in Chapter 2 (section 2.5). A comparison of the findings of this study with those of other studies follows.

7.4.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS

In this study, there was no statistically significant association between ethnic group and ovarian cancer. This is in line with the findings of Firestone et al., who reported that Pacific women had a higher age-standardised incidence rate of ovarian cancer than non-Pacific non-Māori women, but the differences were not statistically significant [197]. Probable reasons for this observation have been discussed in Chapter 2 (section 2.6.1). Use of prioritised ethnicity may have led to misclassification of ethnicity. In addition, non-Pacific non-Māori women are of mixed ethnicity. This may have obscured real differences.

7.4.2 REPRODUCTIVE FACTORS

Findings on parity and timing of deliveries are consistent with those of other studies. An inverse association between parity and the occurrence of ovarian cancer has consistently been shown with lower risk with higher parity [62, 63, 66]. Higher risk in women who had their first pregnancy at ≤19 years compared to those who had their first pregnancy at ≥25 years of age was observed after adjusting for parity [68]. An inverse relationship between age at first delivery and the risk of ovarian cancer has been reported [64]. No association with age at first birth after adjustment for parity and age at last delivery was observed [69]. A statistically significant trend toward lower risk with higher age at last delivery has previously been reported (3%; 95% CI: 1-6%, per year of later age at last delivery) [69], which is consistent with the findings of this study, and as in this study, this was still apparent after adjusting for parity [69].

Breastfeeding has been associated with lower risk of ovarian cancer with a dose-response relationship [76-78]. This inverse association persists after adjustment for parity [76-78]. The relationship between abortion and ovarian cancer is unclear. Statistically non-significant lower risk [83] and no association have both been observed [71]. Inhibition of ovulation may explain reduced risk observed with abortions [71]. OCs have been associated with lower risk of ovarian cancer [63, 83] with a dose-response relationship [83]. The strength of the inverse association has also been shown to be lower with longer duration since last use [7, 240].

Studies assessing the association between age at menarche and the risk of ovarian cancer have had conflicting results: lower risk [46], higher risk [14], and no association [87] have been reported. Higher risk with later age at menopause has previously been observed [46, 83]. Similar to this study, menstrual regularity was not associated with the risk of ovarian cancer in the study of Ness et al. [87].

Previous studies have reported higher risks of ovarian cancer associated with each year of ovulation similar to what we observed in this study (OR = 1.06; 95% CI = 1.04-1.08, and 9.6%; 95% CI = 8.1-11.1) [46, 82]. The lack of an association between ovarian cancer and reproductive factors (parity, age at first and last delivery, and breastfeeding) after adjusting for ovulatory years, suggests that the inverse association with these factors is mainly due to ovulation suppression. On the other hand, the inverse association observed between older age at last delivery and the risk of ovarian cancer, and the persistence of the inverse association with use of OCs points to the likelihood of mechanisms additional to inhibition of ovulation [83].

Estimation of ovulatory years may not be accurate because not all menstrual cycles are ovulatory and this is dependent on age and may vary from woman to woman [80, 82, 83]. Secondly, post-menopausal women may not accurately recall the duration of anovulatory events. In addition, previous studies have truncated the duration of breastfeeding at 6 months per birth [80, 85]. This is because inhibition of ovulation is less effective with longer duration of breastfeeding [80, 85]. We used cumulative months of breastfeeding as opposed to limiting duration of breastfeeding to a maximum of 6 months per birth as others have done, which may have under-estimated the total number of ovulatory years. However, in a study in which lactation was not considered [46] a positive relationship was observed between total ovulatory years and the risk of ovarian cancer. The method used in the current study is the most commonly used in epidemiologic studies and allows for comparison with other studies.

7.4.3 GYNAECOLOGICAL OPERATIONS

In this study, there was no association between ovarian cancer and a history of hysterectomy or oophorectomy. No relationship with age at surgery was observed. History of TL and timing of TL was not associated with ovarian cancer. Findings regarding hysterectomy are consistent with those of other studies [87, 127]. The lack of association between TL and ovarian cancer is at odds with the findings of other studies, in which TL was inversely associated with the risk of ovarian cancer [59, 103, 104]. The lower risk associated with TL

has been shown to differ by histological type of ovarian tumours with the strongest inverse association seen for endometrioid tumours and a weaker inverse association with serous tumours [59, 103]. This has been attributed to their different cells of origin and the extent to which tubal ligation eliminates or prevents these cells from reaching the ovary [59, 103]. The null findings in this study may be because of the large proportion of serous tumours (90 out of 152 [60%]), thought to arise from the distal tubal epithelium, which is not usually removed during tubal ligation. The lack of association with timing of surgery is consistent with the findings of other studies [59, 103]. The lack of association with time since surgery is thought to be due to permanent elimination or obstruction of potential cancer cells from reaching the ovaries [59]. In accord with the findings of our study, a stronger inverse association has previously been reported in women who had had TL and had also used OCs, compared to TL alone [104].

7.4.4 GYNAECOLOGICAL CONDITIONS AND FAMILY HISTORY OF CANCER

In this study, endometriosis and infertility were associated with a statistically significant higher risk of ovarian cancer, whereas no association was observed for history of uterine fibroids or benign ovarian cysts. The association with ovarian cancer observed in women with a history of endometriosis in this study is consistent with the findings of other studies [74, 100, 125]. Similar to the findings of our study, most studies have reported a statistically significantly higher risk of ovarian cancer in women with infertility [88-91], although others have reported no association [92, 93]. Differences in risk according to cause of infertility have also been observed [88, 93]. In this study, information about the cause of infertility was not collected and, therefore, this could not be evaluated.

In this study, a statistically significant positive association was observed between any cancer history in the family and ovarian cancer; whereas there was only a borderline association with history of specific cancers in a first-degree relative. This may be attributed to the lack of detailed information on family history of cancer, which is important in determining genetic predisposition. Statistically significant positive association with any cancer history may be attributed to the possibility of preferential recall by cases or may reflect an appropriate recall of cancer in a family member but not the site of the cancer.

7.4.5 USE OF PMH AND OVULATION-INDUCING DRUGS

In this study, ever-use of PMH was positively associated with ovarian cancer, but there was no association with duration of use. Many studies have reported higher risk of ovarian cancer with ever-use of PMH [134, 138, 141]. Although higher risk with long-term compared to

short-term use has been observed [133, 135, 136, 141], a null association with duration of use has also been observed [134]. We did not collect information on type of PMH used, mode of administration, age at onset of use, or time since last use; therefore, these could not be evaluated in relation to risk.

Findings regarding use of fertility drugs have been inconsistent, with most studies reporting no association [88, 90, 94] or higher risk of ovarian cancer [91]. In this study, a suggestive lower risk was observed with longer duration of use of ovulation-inducing drugs. This is in contrast to other studies which have reported either no relationship [88] or a positive dose-response relationship [90, 91]. Higher risk has been observed in women who use fertility drugs and fail to conceive [88, 92]. Longer use may have resulted in pregnancy, thus, perhaps, the lower risk.

7.4.6 LIFESTYLE FACTORS

The findings of this study regarding the association between talcum use and ovarian cancer are in accord with those of other studies that have reported higher risk of ovarian cancer with use of talcum, but with no dose-response relationship [99-101].

In this study, no association with ovarian cancer was found for women with history of alcohol use compared to non-drinkers. Findings regarding alcohol use have been inconsistent. Some studies have reported no association [145, 147], others lower risk [148], whereas others have observed higher risk [146]. The strength of association has also been shown to differ between mucinous and non-mucinous tumours [149] and by type of alcoholic beverage used [147].

No association was observed between smoking and ovarian cancer in either current or past smokers. A positive association was observed with duration of use and an inverse relationship with time since last use, but there was no association with pack-years. Smoking has been consistently associated with a higher risk of mucinous tumours; the relationship with other histological subtypes is less clear [149-153]. A higher risk has also been reported in borderline tumours compared to invasive tumours [150, 151]. The lack of association in this study may be because we did not include borderline tumours and only 7% (10/152) of the tumours were of mucinous type. Findings on duration of use, pack-years, and age at initiation of use have been inconsistent [149, 150, 153].

7.4.7 ANTHROPOMETRIC MEASURES

In this study, higher current weight and BMI were inversely associated with ovarian cancer. No association was observed with greater usual adult weight, or with height, or weight and

BMI at 18 years. The findings on height are similar to those of a hospital-based case-control study in which no association was observed between height and the risk of ovarian cancer [160], although other studies have reported a positive relationship (higher risk) in taller women [158, 159, 167]. Regarding BMI at 18 years, higher risk [164, 165] and no association [159, 163] have been reported.

Studies that have assessed the association between recent weight/BMI and the risk of ovarian cancer, and observed a positive association were either prospective cohort studies (that used weight/BMI at baseline) [158, 163], or case-control studies that obtained weights 5 years before diagnosis [165]. Some prospective studies have reported no association [157, 159]. The inverse relationship between current weight and the risk of ovarian cancer may be attributed to reverse causation: the average time from diagnosis to participation was five months and participants may have lost weight due the effects of the disease and treatment.

Misclassification error might have occurred in this study because values for weight and height were self-reported and it is also difficult to remember weight in early adulthood. In addition, under-reporting of weight to suit socially desirable values is a possible risk and weight loss by cases due to ovarian cancer is also a possibility. Both of these may have biased the results towards the null.

7.5 CONCLUSION

The consistency of the findings of this study with those of other studies, particularly for parity, breastfeeding, and use of oral contraceptives, which show well-established inverse associations with ovarian cancer, improves confidence in the validity of the findings of this study. The main objective of this study was to assess the association of ever-use of DMPA, vasectomy, and IUDs and the risk of ovarian cancer, which will be covered in Chapters 8-10.

CHAPTER 8: DMPA AND THE RISK OF OVARIAN CANCER

8.1 INTRODUCTION

From the discussion in Chapter 4, it is evident that, although use of DMPA, a long-acting progestogen-based contraceptive, is expected to be inversely associated with ovarian cancer, an association between DMPA and the risk of ovarian cancer has not been established. Previous studies have reported both higher [10, 11] and lower [12, 13] risk of ovarian cancer in users of DMPA. This Chapter covers one of the main objectives of this study, which was to assess the association between ever-use - and specifics of use of DMPA - and the risk of ovarian cancer.

8.2 METHODS

A detailed discussion of study methods is provided in Chapter 5.

Participants were asked whether they had ever-used DMPA, age at first use, time since last use, and duration of use of DMPA. Data were analysed using the IBM Statistical Package for the Social Sciences (IBM SPSS Statistics 22). The association between use of DMPA and specifics of use and ovarian cancer, adjusted for age in five-year groups, were analysed using the Mantel and Haenszel method [290]. When adjusting for more than one variable, binary logistic regression was used. Trend tests were done using actual values rather than categorical values. Odds ratios, 95% confidence intervals, and p-values were calculated.

Ever-use and specifics of use of DMPA were also adjusted for: ever-use of OCs; ever-use of PMH; history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (grouped into: ≤25, 26-30, 31-35, and >35 years); parity (grouped into 1, 2, 3, and ≥4); history of infertility; and age in 5-year groups. These factors were included because they had the strongest association with ovarian cancer, are known risk factors (either higher or lower risk) for ovarian cancer, and each had a statistically significant independent association when entered together into the logistic regression model. In Chapter 7, association with parity, ages at first and last delivery, breastfeeding and use of OCs, condoms, and tubal ligation were adjusted for total ovulatory years. However, in Chapters 8 to 10, this is not done. This is because in multivariate analysis, ever-use and specifics of use of DMPA, IUDs, and vasectomy were adjusted for ever-use of OCs and parity, which were the major determinants of ovulatory years.

Analyses of ever-use of DMPA restricted to women who had never-used and those who had ever-used OCs were also done.

8.3 RESULTS

Only 13 cases (8.6%) and 88 controls (11.8%) were ever-users of DMPA.

8.3.1 AGE-ADJUSTED ANALYSIS

Compared to never-users and adjusting for age (Table 8.1), ever-use of DMPA was associated with an OR of 0.70 (95% CI = 0.38-1.30) for ovarian cancer. Women who first used DMPA when they were >25 years of age had an OR of 0.53 (95% CI = 0.19-1.50), whereas those who were ≤20 years of age at first use had an OR of 0.94 (95% CI = 0.34-2.57).

Duration of use was not statistically significantly associated with ovarian cancer; although use of DMPA for <1, 1-5, and >5 years were associated with ORs of 0.97 (95% CI = 0.32-2.88), 0.68 (95% CI = 0.27-1.66), and 0.40 (95% CI = 0.09-1.68), respectively. Participants who last used DMPA ≤15 years ago had an OR of 0.52 (95% CI = 0.12-2.24), whereas an OR of 0.90 (95% CI = 0.39-2.07) was observed in women who had stopped using DMPA >25 years ago. The risk associated with current use could not be assessed because only four controls and none of the cases were using DMPA at the time of interview. No statistically significant trends were observed for age at first use, duration of use, or time since last use.

8.3.2 MULTIVARIATE ANALYSIS

In multivariate analysis (adjusted for age; ever-use of OCs; ever-use of PMH; parity; age at last delivery; history of infertility; and history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative), overall, there were no changes in the odds ratios (Table 8.1). No changes in risk estimates were observed after further adjustment for ever-use of IUDs and vasectomy (Table 8.1).

Table 8.1: Risk of ovarian cancer associated with use of DMPA compared to never-use

	Cases	Controls	OR[1]	95% CI		P-	OR[2]	95% CI		P-	OR[3]	95% CI		P-
	No. (%)[4]	*No. (%)*[4]		*Lower*	*Upper*	Value		*Lower*	*Upper*	Value		*Lower*	*Upper*	Value
Ever-Use														
No	139 (91)	657 (88)	1.00				1.00				1.00			
Yes	13 (9)	88 (12)	0.70	0.38	1.30	0.328	0.71	0.36	1.39	0.313	0.71	0.36	1.39	0.322
Age 1st Use														
Never Use	139 (92)	657 (88)	1.00				1.00				1.00			
≤20 (11-20)	5 (3)	26 (3)	0.94	0.34	2.57	0.899	0.95	0.33	2.73	0.920	0.94	0.32	2.72	0.937
21-25	3 (2)	26 (3)	0.56	0.16	1.93	0.512	0.60	0.17	2.11	0.424	0.60	0.17	2.11	0.422
>25 (26-43)	4 (3)	35 (5)	0.53	0.19	1.50	0.309	0.45	0.13	1.55	0.208	0.47	0.14	1.60	0.227
Trend Test – per year			*0.988*	*0.896*	*1.090*	*0.813*	*0.940*	*0.803*	*1.101*	*0.444*	*0.947*	*0.810*	*1.108*	*0.497*
Years Since Last Use														
Never Use	139 (91)	657 (89)	1.00				1.00				1.00			
≤15 (0-15)	2 (1)	20 (3)	0.52	0.12	2.24	0.547	0.59	0.13	2.73	0.497	0.60	0.13	2.76	0.508
16-25	4 (3)	19 (3)	0.86	0.28	2.63	0.998	1.09	0.33	3.54	0.892	1.08	0.33	3.53	0.896
>25 (26-50)	7 (5)	40 (5)	0.90	0.39	2.07	0.959	0.79	0.31	2.01	0.624	0.80	0.31	2.02	0.631
Trend Test – per year			*1.035*	*0.962*	*1.113*	*0.353*	*1.072*	*0.953*	*1.206*	*0.248*	*1.066*	*0.942*	*1.207*	*0.248*
Years of Use														
Never Use	139 (92)	657 (89)	1.00				1.00				1.00			
<1 (0)	4 (3)	21 (3)	0.97	0.32	2.88	0.831	1.17	0.37	3.67	0.787	1.12	0.36	3.53	0.841
1-5	6 (4)	40 (5)	0.68	0.27	1.66	0.520	0.64	0.18	2.24	0.482	0.62	0.23	1.68	0.347
>5 (6-30)	2 (1)	24 (3)	0.40	0.09	1.68	0.299	0.56	0.12	2.74	0.476	0.38	0.08	1.72	0.208
Trend Test – per year			*0.936*	*0.811*	*1.081*	*0.369*	*0.869*	*0.680*	*1.111*	*0.263*	*0.862*	*0.647*	*1.149*	*0.311*

[1]Adjusted for age in five-year groups

[2]Adjusted for age in five-year groups; PMH (ever-use); OCs (ever-use); Parity (grouped into: 1, 2, 3, and ≥4); history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (grouped into: ≤25, 26-30, 31-35, and >35); history of infertility.

[3]Additionally adjusted for ever-use of IUDs and vasectomy.

[4]Percentages are of total stated

8.3.3 ANALYSIS WITH WOMEN WHO HAVE NEVER-USED DMPA/OCs AS THE REFERENCE GROUP

Use of OCs may have influenced the association of DMPA with the risk of ovarian cancer. In an attempt to disentangle the association of DMPA from that of OCs, women who had used neither OCs nor DMPA were used as the reference group (Table 8.2). There were 34 cases and 70 controls who had not used DMPA or OCs. When these women were used as the reference group, and adjusting for age, a statistically significant inverse association with ever-use of DMPA was seen (OR = 0.26; 95% CI = 0.12-0.55). There were no changes in the relationship with age at first use, time since last use, and duration of use. In multivariate analysis, ever-use of DMPA was associated with an OR of 0.50 (95% CI = 0.08-3.10), and there were no changes in the relationship with age at first use, time since last use, or duration of use.

Table 8.2: Risk of ovarian cancer associated with use of DMPA compared to use of neither DMPA nor OCs

	Cases	**Controls**	**OR[1]**	**95% CI**		**P-**	**OR[2]**	**95% CI**		**P-**
	No. (%)[3]	***No. (%)[3]***		***Lower***	***Upper***	**Value**		***Lower***	***Upper***	**Value**
Ever-Use										
No	34 (72)	70 (44)	1.00				1.00			
Yes	13 (28)	88 (56)	0.26	0.12	0.55	0.001	0.50	0.08	3.10	0.454
Age 1st Use										
Never	34 (74)	70 (45)	1.00				1.00			
≤20 (11-20)	5 (11)	26 (17)	0.31	0.10	1.00	0.088	0.60	0.09	4.03	0.597
21-25	3 (7)	26 (17)	0.20	0.05	0.80	0.035	0.34	0.03	3.30	0.350
>25 (26-43)	4 (9)	35 (22)	0.24	0.08	0.69	0.009	0.14	0.01	1.91	0.140
Trend test – per year							*0.940*	*0.803*	*1.101*	*0.444*
Years Since Last Use										
Never	34 (72)	70 (47)	1.00				1.00			
≤15 (0-15)	2 (4)	20 (13)	0.21	0.05	0.96	0.055	0.24	0.02	3.54	0.301
16-25	4 (9)	19 (13)	0.26	0.07	0.94	0.065	1.21	0.12	12.49	0.875
>25 (26-50)	7 (15)	40 (27)	0.40	0.15	1.04	0.094	1.13	0.13	9.53	0.875
Trend test – per year							*1.072*	*0.953*	*1.203*	*0.248*
Years of Use										
Never	34 (74)	70 (45)	1.00				1.00			
<1 (0)	4 (9)	21 (14)	0.40	0.13	1.29	0.192	1.03	0.12	9.08	0.981
1-5	6 (13)	40 (26)	0.21	0.07	0.61	0.007	0.46	0.06	3.36	0.442
>5 (6-30)	2 (4)	24 (15)	0.15	0.03	0.68	0.013	0.33	0.04	3.04	0.329
Trend test – per year							*0.869*	*0.680*	*1.111*	*0.263*

[1]Adjusted for age in five-year groups

[2]Adjusted for age in five-year groups; PMH (ever-use); OCs (ever-use); Parity (grouped into: 1, 2, 3, and ≥4); history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (grouped into: ≤25, 26-30, 31-35, and >35); history of infertility.

[3]Percentages are of total stated

8.3.4 ANALYSES RESTRICTED TO EVER- AND NEVER-USERS OF OCs

In an analysis restricted to ever-users of OCs, ever-use of DMPA was associated with an OR of 0.78 (95% CI = 0.40-1.52; Table 8.3), which was not different from that observed for ever- versus never-use in all the participants (Table 8.1). Among DMPA ever-users, only 2 cases and 7 controls had never used OCs. When this group of women was compared to women who had used neither OCs nor DMPA, an OR of 0.42 (95% CI = 0.07-2.52) was observed (Table 8.3). For these restricted analyses, the numbers were too small to allow for assessment of specifics of use.

Table 8.3: Risk of ovarian cancer in ever-users of DMPA compared to never-users in women who have ever-used oral contraceptives and in those who have never-used oral contraceptives

		Cases	Controls	OR[1]	95% CI		P-Value	OR[2]	95% CI		P-Value
		No. (%)[3]	*No. (%)*[3]		*Lower*	*Upper*			*Lower*	*Upper*	
Ever-users of oral contraceptives											
DMPA Use											
	Never	105 (91)	587 (88)	1.00							
	Ever	11 (9)	81 (12)	0.78	0.40	1.52	0.569	0.74	0.36	1.52	0.412
Never-users of oral contraceptives											
DMPA Use											
	Never	34 (94)	70 (91)	1.00				1.00			
	Ever	2 (6)	7 (9)	0.42	0.07	2.52	0.591	0.39	0.06	2.63	0.332

[1]Adjusted for age in five-year groups

[2]Adjusted for age in five-year groups; PMH (ever-use); OCs (ever-use); Parity (grouped into: 1, 2, 3, and ≥4); history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (grouped into: ≤25, 26-30, 31-35, and >35); history of infertility.

[3]Percentages are of total stated

8.3.5 RISK OF OVARIAN CANCER ASSOCIATED WITH EVER-USE OF DMPA BY HISTOLOGICAL TYPE

Risk for serous and non-serous tumours was assessed in relation to ever-use of DMPA, adjusted for age in 5-year groups (Table 8.4). Compared to never-use, no difference in risk associated with ever-use of DMPA was observed between serous and non-serous tumours (OR = 0.70 for both).

Risk for mucinous and non-mucinous tumours was also assessed, adjusted for age (categorised into <60 years and ≥60 years due to the small number of cases with mucinous tumours) (Table 8.4). Compared to never-use, ever-use of DMPA was associated with an OR of 1.89 (95% CI = 0.39-9.18) for mucinous tumours and an OR of 0.60 (95% CI = 0.30–1.19) for non-mucinous tumours.

Table 8.4: Risk of ovarian cancer associated with ever-use of DMPA by histological type

	Cases *No. (%)*[1]	Controls *No. (%)*[1]	OR	95% CI *Lower Upper*	P-value
	Serous[2]				
DMPA Use					
Never	83 (92)	657 (88)	1.00		
Ever	7 (8)	88 (12)	0.70	0.31 1.58	0.502
	Non-serous[2]				
DMPA Use					
Never	47 (90)	657 (88)	1.00		
Ever	5 (10)	88 (12)	0.70	0.27 1.83	0.614
	Mucinous[3]				
DMPA Use					
Never	8 (80)	657 (88)	1.00		
Ever	2 (20)	88 (12)	1.89	0.39 9.18	0.763
	Non-Mucinous[3]				
DMPA Use					
Never	122 (92)	657 (88)	1.00		
Ever	10 (8)	88 (12)	0.60	0.30 1.19	0.185

[1]Percentages are of total stated
[2]ORs adjusted for age in five-year groups
[3]ORs adjusted for age (categorised into <60 and ≥60 years).

8.4 DISCUSSION

A detailed discussion of previous studies assessing the association between use of DMPA and the risk of ovarian cancer and possible biologic mechanisms of this association has already been provided in Chapter 4 (sections 4.4.3 and 4.5.3). A comparison of the findings of this study with those of other studies follows.

Apart from the statistically significant inverse association in an analysis with those unexposed both to DMPA and OCs as comparison (OR = 0.26; 95% CI = 0.12-0.55), this study did not demonstrate a statistically significant association between DMPA and ovarian cancer but this is plausibly related to the low power of the study (discussed in Chapter 11-section 11.2.2.1). In order to compare the findings of this study with other studies of DMPA and ovarian cancer, some of which had similarly low power [10-12], the direction of associations will be compared. Although it would be unwise to place too much weight on results that are not statistically significant, a consistent direction of association in many studies may point to a causal association. In this study, use of DMPA was associated with odds ratios below 1.00. In addition, a differential result for non-mucinous and mucinous tumours was observed. This is similar to what is observed with use of OCs and, again, may strengthen causal inference. Furthermore, a stronger inverse association with initiation of use at an older age would be similar to the observation that older age at last delivery is associated

with a lower risk of ovarian cancer and perhaps points to a shared mechanism of action, such as clearance of malignant epithelial cells mediated by high progesterone levels.

Previous studies have reported a statistically significant inverse association between use of DMPA and the risk of ovarian cancer [12, 13]. One of these studies had a higher number of ever-users of DMPA (59 cases and 252 controls) [13], whereas the other study had a similar number of ever-users of DMPA as the current study (14 cases and 141 controls) [12]. However, the latter study did not differentiate between combined injectable contraceptives and progestogen-only injectable contraceptives [12].

A hospital-based case-control study carried out in Thailand also reported a duration-response relationship [13]. Use of DMPA for ≤1 year, 2 years, 3 years, and >3 years were associated with ORs of 0.96, 0.86, 0.39, and 0.17 respectively, although only use for >3 years was associated with a statistically significant lower risk [13]. Earlier studies observed statistically non-significant risk estimates greater than 1.00 for ovarian cancer associated with use of DMPA [10, 11]; OR = 1.1 (95% CI = 0.6-1.8) [10] and OR = 2.8 (95% CI = 0.9-8.5) [11]. However, these studies were carried out when DMPA had been used for only a short time [13].

The WHO Collaborative Study of Neoplasia and Steroid Contraceptives reported no difference in risk across the histological types (serous, mucinous and endometrioid ovarian cancer [P = 0.77]) [10]. However, consistent with this study, that study found different directions of association for mucinous tumours (RR = 1.4; 95% CI = 0.7–2.9) and serous tumours (RR = 0.8; 95% CI = 0.3–2.7) [10].

The findings of this study and those of other studies [12, 13] suggest an inverse association between ovarian cancer and use of DMPA, which forms an additional important non-contraceptive benefit of use of DMPA. In addition, this sheds additional light on possible pathogenic mechanisms of ovarian cancer.

CHAPTER 9: IUDS AND THE RISK OF OVARIAN CANCER

9.1 INTRODUCTION

Studies assessing the association between use of IUDs and the risk of ovarian cancer have reported both higher [7, 241] and lower risk [11, 14]. However, the findings of two of these studies were not statistically significant [11, 241]. This Chapter addresses one of the main objectives of this study, which was to assess the association between ever-use and specifics of use of IUDs and the risk of ovarian cancer.

9.2 METHODS

Study methods were discussed in detail in Chapter 5.

Participants were asked whether they had ever-used an IUD, age at first use, time since last use, duration of use, and type(s) of IUD(s) used. Choices given for IUD types included copper T/copper 7, Multiload, Mirena, others, and don't know. Data were analysed using the IBM Statistical Package for the Social Sciences (IBM SPSS Statistics 22). The association of ever-use of IUDs and patterns of use (age at first use, duration of use, and time since last use), were assessed, adjusting for age in 5-year groups, using the Mantel and Haenszel method [290]. When adjusting for more than one variable, binary logistic regression was used. Trend tests were done using actual values rather than categorical values. Odds ratios, 95% confidence intervals, and p-values were calculated.

Ever-use and specifics of use of IUDs were also adjusted for ever-use of oral contraceptives; ever-use of PMH; history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (grouped into ≤25, 26-30, 31-35, and >35); parity (grouped into 1, 2, 3, and ≥4); history of infertility; and age in 5-year groups. These factors were included because they had the strongest association with ovarian cancer, are known risk factors (either higher or lower risk) for ovarian cancer, and each had a statistically significant independent association when entered together into the logistic regression model.

Additional adjustment for ever-use of DMPA and vasectomy was done. Analyses of the association between use of IUDs and the risk of ovarian cancer restricted to women who had never-used and those who had ever-used hormonal contraceptives (OCs or DMPA) were also done.

9.3 RESULTS

In this study, 40 (26.3%) cases and 191 (25.6%) controls had ever used IUDs.

9.3.1 AGE-ADJUSTED ANALYSIS

Relative to never-use, ever-use of IUDs was not associated with ovarian cancer in age-adjusted analysis (OR = 0.98; 95% CI = 0.66-1.47) (Table 9.1). There was also no relationship with age at first use (P-trend = 0.735). Lower risk with longer time since last use was observed (P-trend = 0.032). Women who had stopped using an IUD within one year before diagnosis, had an OR of 8.26 (95% CI = 2.11-32.33), whereas those who last used IUDs >30 years ago had an OR of 0.39 (95% CI = 0.15-1.00). Longer duration of use was associated with higher risk (P-trend = 0.030). Users of IUDs for 1-5 years had an OR of 0.82 (95% CI = 0.46-1.45), whereas an OR of 3.21 (95% CI = 0.98-10.53) was observed in those who had used IUDs for >20 years.

There was no statistically significant association between type of IUD used compared to never-use of IUDs, and ovarian cancer. Analysis of 'other' IUDs was not done because only two controls and none of the cases had used 'other' IUDs.

Table 9.1: Risk of ovarian cancer associated with use of IUDs compared to never-use

	Cases	Controls	OR[1]	95% CI		P-Value	OR[2]	95% CI		P-Value	OR[3]	95% CI		P-Value
	No. (%)[4]	*No. (%)*[4]		*Lower*	*Upper*			*Lower*	*Upper*			*Lower*	*Upper*	
Ever-Use														
No	112 (74)	554 (74)	1.00				1.00				1.00			
Yes	40 (26)	191 (26)	0.98	0.66	1.47	0.982	1.25	0.81	1.93	0.309	1.25	0.81	1.93	0.315
Age 1st Use														
Never Used	112 (74)	554 (75)	1.00				1.00				1.00			
≤20 (16-20)	4 (3)	15 (2)	1.07	0.34	3.36	0.855	1.86	0.57	6.10	0.306	1.86	0.57	6.08	0.307
21-25	12 (8)	63 (8)	0.86	0.45	1.67	0.784	1.10	0.56	2.19	0.780	1.10	0.56	2.19	0.781
26-30	9 (6)	50 (7)	0.87	0.41	1.83	0.852	1.07	0.49	2.35	0.868	1.08	0.49	2.38	0.840
31-35	7 (5)	22 (3)	1.68	0.70	4.04	0.364	1.98	0.71	5.48	0.189	1.96	0.70	5.46	0.198
>35 (36-51)	7 (5)	39 (5)	0.79	0.34	1.83	0.726	1.03	0.43	2.48	0.947	1.01	0.42	2.44	0.981
Trend Test – per year			*0.992*	*0.945*	*1.041*	*0.735*	*0.980*	*0.930*	*1.032*	*0.444*	*0.979*	*0.929*	*1.032*	*0.440*
Years Since Last Use														
Never Used IUDs	112 (76)	554 (78)	1.00				1.00				1.00			
<1 (0)	6 (4)	3 (0.4)	8.26	2.11	32.33	0.001	11.71	2.69	52.15	0.001	12.30	2.74	55.12	0.001
1-5	7 (5)	15 (2)	1.84	0.72	4.71	0.310	2.20	0.812	5.98	0.121	2.25	0.82	6.12	0.114
6-10	3 (2)	7 (1)	1.80	0.45	7.14	0.659	2.13	0.47	9.69	0.329	2.06	0.44	9.59	0.355
11-20	4 (3)	24 (3)	0.76	0.26	2.24	0.805	0.88	0.28	2.72	0.818	0.82	0.26	2.57	0.737
21-30	11 (7)	47 (7)	1.02	0.50	2.08	0.903	1.22	0.57	2.60	0.611	1.25	0.58	2.68	0.567
>30 (31-44)	5 (3)	63 (9)	0.39	0.15	1.00	0.065	0.53	0.20	1.39	0.196	0.54	0.20	1.41	0.205
Trend Test – per year			*0.959*	*0.923*	*0.996*	*0.032*	*0.956*	*0.915*	*0.999*	*0.043*	*0.961*	*0.920*	*1.005*	*0.081*
Years of Use														
Never Used IUDs	112 (75)	554 (75)	1.00				1.00				1.00			
<1 (0)	5 (3)	25 (3)	0.94	0.34	2.42	0.967	1.32	0.47	3.72	0.601	1.33	0.47	3.77	0.588
1-5	16 (11)	93 (13)	0.82	0.46	1.45	0.574	0.96	0.52	1.78	0.891	0.97	0.52	1.81	0.932
6-10	7 (5)	41 (6)	0.76	0.33	1.76	0.657	1.21	0.51	2.89	0.666	1.19	0.50	2.84	0.702
11-20	4 (3)	19 (3)	0.91	0.30	2.70	0.925	1.05	0.33	3.29	0.940	1.01	0.32	3.19	0.987
>20 (21-40)	5 (3)	7 (1)	3.21	0.98	10.53	0.103	3.09	0.89	10.77	0.076	2.87	0.82	10.04	0.099
Trend Test – per year			*1.056*	*1.005*	*1.110*	*0.030*	*1.059*	*1.003*	*1.118*	*0.040*	*1.054*	*0.996*	*1.116*	*0.069*
IUD Type														
Never Used IUDs	112 (74)	554 (74)	1.00				1.00				1.00			
Copper T or 7	18 (12)	100 (13)	0.85	0.49	1.47	0.662	1.13	0.63	2.02	0.678	1.13	0.63	2.02	0.684
Multiload	2 (1)	11 (1)	0.75	0.16	3.54	0.997	1.07	0.21	5.38	0.940	1.10	0.22	5.53	0.912
Mirena	8 (5)	45 (6)	0.73	0.33	1.64	0.572	0.91	0.38	2.18	0.828	0.89	0.37	2.13	0.787
Don't Know	13 (9)	47 (6)	1.32	0.69	2.52	0.510	1.53	0.75	3.13	0.241	1.55	0.76	3.18	0.228

[1]Adjusted for age in five-year groups

[2]Adjusted for age in five-year groups; PMH (ever-use); OCs (ever-use); Parity (grouped into: 1, 2, 3, and ≥4); history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (grouped into: ≤25, 26-30, 31-35, and >35); history of infertility.

[3]Additionally adjusted for ever-use of DMPA, and vasectomy. [4]Percentages are of total stated

9.3.2 MULTIVARIATE ANALYSIS

Ever-use of IUDs and particulars of use (age at first use, duration of use, time since last use, and type of IUDs ever used) were also adjusted for: age; ever-use of OCs; ever-use of PMH; parity; age at last delivery; history of infertility; and history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative (Table 9.1). Ever- compared to never-use of IUDs was not associated with ovarian cancer (OR = 1.25; 95% CI = 0.81-1.93). No relationship was observed with age at first use (P-trend = 0.444). A statistically significant trend toward higher risk with longer duration of use was observed (P-trend = 0.040). Highest risk was observed with use of IUDs for >20 years (OR = 3.09; 95% CI = 0.89-10.77). Time since last use was associated with a lower risk (P-trend = 0.043).

Ever-use of copper T/ copper 7, Multiload, or Mirena were not associated with ovarian cancer. No changes in risk estimates were observed after adjustment for ever-use of DMPA and vasectomy.

9.3.3 ANALYSIS WITH WOMEN WHO HAVE USED NEITHER HORMONAL CONTRACEPTIVES (DMPA/OCS) NOR IUDS AS THE REFERENCE GROUP

Among never-users of IUDs, 29 cases and 59 controls had also never used OCs or DMPA. When these participants were used as the reference group, ever-use of IUDs was associated with a statistically significant lower risk in age-adjusted analysis (OR = 0.41; 95% CI= 0.23-0.73), but the association did not persist in multivariate analysis (Table 9.2). No change was observed in the relationship between risk of ovarian cancer and age at first use, time since last use, and duration of use.

In multivariate analysis, no association was observed in ever- compared to never-users of IUDs (OR = 1.45; 95% CI = 0.40-5.30). No association was observed with age at first use. Higher risk with more years of use was observed (P-trend = 0.040). Longer time since last use was associated with a lower risk (P-trend = 0.043).

Table 9.2: Risk of ovarian cancer associated with use of IUDs compared to users of neither IUDs nor hormonal contraceptives (DMPA; oral contraceptives)

	Cases		Controls		OR[1]	95% CI		P-Value	OR[2]	95% CI		P-Value
	No.	*(%)*[3]	*No.*	*(%)*[3]		*Lower*	*Upper*			*Lower*	*Upper*	
Ever-Use												
No (None)	29	(42)	59	(24)	1.00				1.00			
Yes	40	(58)	191	(76)	0.41	0.23	0.73	0.004	1.45	0.40	5.30	0.574
Age 1st Use												
Never Use	29	(43)	59	(24)	1.00				1.00			
≤20 (16-20)	4	(6)	15	(6)	0.56	0.14	2.20	0.618	3.48	0.55	21.99	0.184
21-25	12	(18)	63	(25)	0.37	0.16	0.85	0.028	1.44	0.35	5.89	0.610
26-30	9	(13)	50	(20)	0.34	0.14	0.83	0.029	1.33	0.31	5.71	0.701
31-35	7	(10)	22	(9)	0.64	0.24	1.71	0.518	2.11	0.40	11.04	0.376
>35 (36-51)	7	(10)	39	(16)	0.29	0.11	0.78	0.021	1.08	0.23	4.97	0.924
Trend Test – per year					*0.992*	*0.945*	*1.041*	*0.735*	*0.980*	*0.930*	*1.032*	*0.444*
Years Since Last Use												
Never Use	29	(45)	59	(27)	1.00							
<1 (0)	6	(9)	3	(1)	2.77	0.58	13.12	0.363	5.86	0.77	44.58	0.088
1-5	7	(11)	15	(7)	0.65	0.20	2.04	0.647	1.34	0.24	7.49	0.742
6-10	3	(5)	7	(3)	0.75	0.18	3.20	0.976	1.63	0.20	13.49	0.649
11-20	4	(6)	24	(11)	0.30	0.09	1.03	0.094	0.64	0.09	4.28	0.624
21-30	11	(17)	47	(22)	0.45	0.18	1.12	0.140	0.85	0.16	4.48	0.852
>30 (31-44)	5	(8)	63	(29)	0.17	0.06	0.50	0.001	0.33	0.05	2.11	0.242
Trend Test – per year					*0.959*	*0.923*	*0.996*	*0.032*	*0.956*	*0.915*	*0.999*	*0.043*
Years of Use												
Never Use	29	(44)	59	(24)	1.00							
<1 (0)	5	(8)	25	(10)	0.39	0.12	1.24	0.179	1.20	0.20	7.16	0.844
1-5	16	(24)	93	(38)	0.32	0.15	0.67	0.004	0.74	0.16	3.42	0.696
6-10	7	(11)	41	(17)	0.28	0.10	0.75	0.017	0.84	0.16	4.48	0.839
11-20	4	(6)	19	(8)	0.35	0.10	1.20	0.153	0.83	0.14	4.75	0.829
>20 (21-40)	5	(8)	7	(3)	1.38	0.37	5.22	0.898	3.620	0.66	20.01	0.140
Trend Test – per year					*1.056*	*1.005*	*1.110*	*0.030*	*1.059*	*1.003*	*1.118*	*0.040*
IUD Type												
Never Used	29	(41)	59	(23)	1.00				1.00			
Copper T or 7	18	(26)	100	(38)	0.36	0.18	0.74	0.008	0.24	0.02	3.26	0.285
Multiload	2	(3)	11	(4)	0.28	0.05	1.47	0.206	-	-	-	-
Mirena	8	(11)	45	(17)	0.21	0.07	0.62	0.007	4.18	0.15	116.95	0.400
Don't Know	13	(19)	47	(18)	0.58	0.26	1.28	0.248	4.14	0.53	32.39	0.175

[1]Adjusted for age in five-year groups

[2]Adjusted for age in five-year groups; PMH (ever-use); OCs (ever-use); Parity (grouped into: 1, 2, 3, and ≥4); history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (grouped into: ≤25, 26-30, 31-35, and >35); history of infertility.

[3]Percentages are of total stated

9.3.4 ANALYSIS RESTRICTED TO EVER-USERS OF HORMONAL CONTRACEPTIVES (DMPA/OCS)

In an analysis confined to ever-users of hormonal contraceptives (Table 9.3), ever-use of IUDs was not associated with the risk of ovarian cancer (OR = 1.09; 95% CI = 0.71-1.68). No relationship with age at first use was observed (P-trend = 0.764). No association between risk and duration of use was observed. Lower risk was observed with longer duration since last use (P-trend = 0.027). Use of any specific type of IUD was not associated with ovarian cancer. Similar observations were made in multivariate analysis.

Table 9.3: Risk of ovarian cancer associated with use of IUDs compared to never-use, among ever-users of hormonal contraceptives (DMPA or OCs)

	Cases		Controls		OR[1]	95% CI		P-	OR[2]	95% CI		P-
	No.	*(%)*[3]	*No.*	*(%)*[3]		*Lower*	*Upper*	Value		*Lower*	*Upper*	Value
Ever-Use												
No	83	(70)	495	(73)	1.00				1.00			
Yes	35	(30)	180	(27)	1.09	0.71	1.68	0.772	1.14	0.72	1.81	0.572
Age 1st Use												
Never Used	83	(71)	495	(74)	1.00				1.00			
≤20 (16-20)	3	(3)	15	(2)	0.90	0.25	3.19	0.886	1.25	0.33	4.74	0.748
21-25	11	(9)	59	(9)	0.98	0.49	1.95	0.909	0.99	0.47	2.07	0.972
26-30	7	(6)	49	(7)	0.83	0.36	1.90	0.805	0.93	0.40	2.17	0.861
31-35	6	(5)	21	(3)	1.84	0.72	4.69	0.309	1.97	0.74	5.22	0.173
>35 (36-51)	7	(6)	34	(5)	1.10	0.47	2.57	0.989	1.06	0.42	2.70	0.901
Trend Test – per year					*1.000*	*0.999*	*1.000*	*0.764*	*0.996*	*0.943*	*1.051*	*0.876*
Years Since Last Use												
Never-Use	83	(72)	495	(77)	1.00				1.00			
<1 (0)	4	(3)	3	(0.5)	6.78	1.61	28.53	0.008	9.61	1.94	47.57	0.006
1-5	7	(6)	13	(2)	2.51	0.95	6.59	0.105	3.30	1.18	9.17	0.022
6-10	3	(3)	6	(1)	2.39	0.58	9.79	0.418	1.83	0.34	9.72	0.478
11-20	4	(3)	23	(4)	0.93	0.31	2.76	0.886	0.86	0.28	2.71	0.802
21-30	9	(8)	45	(7)	1.00	0.46	2.15	0.850	1.09	0.48	2.51	0.836
>30 (31-44)	5	(4)	60	(9)	0.48	0.19	1.24	0.174	0.62	0.23	1.65	0.340
Trend Test – per year					*0.95*	*0.91*	*.99*	*0.027*	*0.958*	*0.914*	*1.005*	*0.077*
Years of Use												
Never-Use	83	(72)	495	(74)	1.00				1.00			
<1 (0)	5	(4)	25	(4)	1.04	0.39	2.79	0.865	1.25	0.44	3.53	0.676
1-5	15	(13)	88	(13)	0.96	0.53	1.74	0.993	1.07	0.57	2.01	0.843
6-10	7	(6)	38	(6)	0.98	0.41	2.31	0.868	0.91	0.36	2.30	0.844
11-20	3	(3)	18	(3)	0.86	0.25	2.97	0.950	0.77	0.21	2.83	0.693
>20 (21-40)	3	(3)	5	(1)	2.97	0.71	12.38	0.262	3.64	0.79	16.78	0.097
Trend Test – per year					*1.05*	*0.99*	*1.11*	*0.097*	*1.04*	*0.979*	*1.108*	*0.193*
IUD Type												
Never-Use	83	(71)	495	(72)	1.00				1.00			
Copper T or 7	16	(14)	95	(14)	0.95	0.53	1.69	0.978	0.94	0.51	1.74	0.851
Multiload	1	(1)	10	(1)	0.48	0.06	4.02	0.790	0.35	0.04	3.09	0.344
Mirena	7	(6)	42	(6)	0.84	0.37	1.98	0.854	0.88	0.36	2.15	0.783
Don't Know	10	(9)	43	(6)	1.29	0.63	2.66	0.614	1.73	0.798	3.739	0.166
Never used hormonal contraceptives (DMPA/OCs)												
IUD ever-use												
No	29	(85)	59	(84)	1.00				1.00			
Yes	5	(15)	11	(16)	1.03	0.31	3.41	0.799	3.33	0.61	18.32	0.166

[1]Adjusted for age in five-year groups

[2]Adjusted for age in five-year groups; PMH (ever-use); OCs (ever-use); Parity (grouped into: 1, 2, 3, and ≥4); history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (grouped into: ≤25, 26-30, 31-35, and >35); history of infertility.

[3]Percentages are of total stated

9.3.5 ANALYSIS RESTRICTED TO NEVER-USERS OF HORMONAL CONTRACEPTIVES (DMPA/OCS)

Among ever-users of IUDs, 5 cases and 11 controls had never used OCs or DMPA. In analyses restricted to never-users of hormonal contraceptives (Table 9.3, bottom panel), ever-use of IUDs was not associated with the risk of ovarian cancer in the age-adjusted analysis

(OR = 1.03; 95% CI = 0.31-3.41) or in multivariate analysis (OR = 3.33; 95% CI = 0.61-18.32).

9.3.6 RISK OF OVARIAN CANCER ASSOCIATED WITH EVER-USE OF IUDS BY HISTOLOGIC TYPE

Risk of serous and non-serous tumours was assessed in relation to ever-use of IUDs, adjusted for age in 5-year groups (Table 9.4). Relative to never-use, ever-use of IUDs was associated with an OR of 1.04 (95% CI = 0.63–1.72) for serous tumours and, for non-serous tumours an OR of 0.86 (95% CI = 0.44-1.66).

Risk of mucinous and non-mucinous tumours was also assessed, adjusted for age (categorised into <60 years and ≥60 years due to the small number of cases with mucinous tumours) (Table 9.4). Compared to never-use, ever-use of IUDs was associated with an OR of 1.08 (95% CI = 0.71–1.64) for non-mucinous tumours and an OR of 0.32 (95% CI = 0.04-2.56) for mucinous tumours.

Table 9.4: Risk of ovarian cancer associated with ever-users of IUDs by histological type

	Cases *No. (%)*[1]	Controls *No. (%)*[1]	OR	95% CI *Lower Upper*	P-value
	Serous[2]				
IUD Use					
Never	65 (72)	554 (74)	1.00		
Ever	25 (28)	191 (26)	1.04	0.63 1.72	0.979
	Non-serous[2]				
IUD Use					
Never	40 (77)	554 (74)	1.00		
Ever	12 (23)	191 (26)	0.86	0.44 1.66	0.767
	Mucinous[3]				
IUD Use					
Never	9 (90)	554 (74)	1.00		
Ever	1 (10)	191 (26)	0.32	0.04 2.56	0.446
	Non-Mucinous[3]				
IUD Use					
Never	96 (73)	554 (74)	1.00		
Ever	36 (27)	191 (26)	1.08	0.71 1.64	0.801

[1]Percentages are of total stated
[2]Adjusted for age in five-year groups
[3]Adjusted for age (categorised into <60 and ≥60 years).

9.4 DISCUSSION

Previous studies assessing the association between use of IUDs and the risk of ovarian cancer, and possible biologic mechanisms of this association have been discussed in more

detail in Chapter 4 (sections 4.4.4 and 4.5.4). A comparison of the findings of this study with those of other studies follows.

In this study, no association between ever-use of IUDs and the risk of ovarian cancer was observed. A statistically significant positive trend was observed for duration of use and ovarian cancer (P-trend = 0.030), and an inverse association was observed with time since last use (P-trend = 0.032).

Both a statistically significant positive association [7] and inverse associations [11, 14] between ever-use of IUDs and ovarian cancer have been reported. Ness et al. [14] reported lower risk with short duration of use and longer use (albeit statistically non-significantly) was associated with higher risk (ORs for use for ≤4 years, 5-9 years, and ≥10 years were 0.53, 1.11, and 1.40, respectively), which is consistent with the findings of this study.

In addition, similar to the current study, they also observed a trend toward lower risk with longer time since last use, but this was no longer evident after adjustment for duration of use. In the current study, cessation of use within one year of diagnosis may be linked to removal of IUDs at hysterectomy following a diagnosis of ovarian cancer. In addition, the inverse association observed (in age-adjusted analysis) when never-users of OCs and DMPA were used as the reference group is similar to the findings by Ness et al., in which a stronger association was seen when women who had never used any type of contraceptive were used as the reference group [14].

The dose-duration response relationship observed in both this study and that of Ness et al. may suggest a causal association between IUD use and ovarian cancer. Tworoger et al. reported a statistically significant higher risk of ovarian cancer in ever-users of IUDs (RR = 1.76; 95% CI = 1.08-2.85), but with a stronger association for serous (RR = 2.17) and endometrioid (RR = 2.40) tumours [7]. Consistent with the findings of this study, a prospective cohort study reported no statistically significant association between age at initiation of use of IUDs and the risk of ovarian cancer [241].

The findings of this study do not disprove our *a priori* hypothesis that IUDs, particularly copper-bearing IUDs, increase the risk of ovarian cancer. Assessment of risk by type of IUD used was precluded by the small number of ever-users of IUDs and a large proportion of participants (ever-users) who were not aware of the IUD types they had used (13/40 [33%]).

The findings of this study may be ascribed to copper-bearing IUDs because for those who indicated type of IUD used, 74% (20/27) had used such IUDs.

The findings of this study regarding the association between use of IUDs and the risk of ovarian cancer are inconclusive. A study with enough power or a collaborative analysis of existing studies to assess the association between ever-use and risk of ovarian cancer, as well as differences in risk according to IUD type, is needed.

CHAPTER 10: VASECTOMY AND THE RISK OF OVARIAN CANCER

10.1 INTRODUCTION

As discussed in Chapter 4, little research has been done on the association between partner vasectomy and the risk of ovarian cancer. This Chapter addresses one of the main objectives of this study, which was to assess the association between partner vasectomy and duration of reliance on vasectomy for contraception, and ovarian cancer.

10.2 METHODS

A detailed discussion of study methods has already been provided in Chapter 5.

Participants were asked whether they had ever had a partner who has undergone vasectomy, and for how long they relied on vasectomy for contraception. Data were analysed using the IBM Statistical Package for the Social Sciences (IBM SPSS Statistics 22). The associations between ever-use of vasectomy and duration of reliance on vasectomy for contraception and ovarian cancer, adjusted for age in five-year groups, were analysed using the method of Mantel and Haenszel [290]. When adjusting for more than one variable, binary logistic regression was used. Trend tests were done using actual values rather than categorical values. Odds ratios, 95% confidence intervals, and p-values were calculated.

In multivariate analysis, the association between history of ever having had a vasectomised partner and duration of reliance on vasectomy for contraception and the risk of ovarian cancer were also adjusted for ever-use of oral contraceptives; ever-use of PMH; history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (categorised into: ≤25, 26-30, 31-35, and >35); history of infertility; parity (categorised into: 1, 2, 3, and ≥ 4); and age in 5-year groups. These factors were included because they had the strongest association with ovarian cancer, are known risk factors (either higher or lower risk) for ovarian cancer, and each had a statistically significant independent association when entered together into the logistic regression model.

Additional adjustment for ever-use of DMPA and IUDs was done. Analyses of use of vasectomy restricted to women who had never-used and those who had ever-used hormonal contraceptives (oral contraceptives or DMPA) were also done.

10.3 RESULTS

There were 54 (35.5%) cases and 332 (44.5%) controls with a history of ever having had a vasectomised partner.

10.3.1 AGE-ADJUSTED ANALYSIS

A statistically significant lower risk of ovarian cancer was observed in women who had ever - compared to those who had never - had a vasectomised partner in age-adjusted analysis (OR = 0.67, 95% CI = 0.46-0.96) (Table 10.1). Use for more than one year was associated with lower risk, with the lowest risk seen in those who relied on vasectomy for 11- 15 years (OR = 0.27; 95% CI = 0.10 - 0.79). Overall, the strength of the inverse association was greater with longer duration of reliance on vasectomy (P-trend = 0.051).

10.3.2 MULTIVARIATE ANALYSIS

In multivariate analysis (Table 10.1), the association between history of having a vasectomised partner and duration of reliance on vasectomy for contraception and the risk of ovarian cancer were adjusted for: age; ever-use of OCs; ever-use of PMH; parity; age at last delivery; history of infertility; and history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative. The strength of the inverse association was less marked and was no longer statistically significant (OR = 0.82; 95% CI = 0.54-1.23). Findings regarding duration of use were not different from those of age-adjusted analysis. Additional adjustment for ever-use of DMPA and IUDs did not alter the findings.

Table 10.1: Risk of ovarian cancer in women with a history of ever having had a vasectomised partner compared with those with no history of having a vasectomised partner

	Cases		**Controls**		**OR[1]**	**95% CI**		**P-Value**	**OR[2]**	**95% CI**		**P-Value**	**OR[3]**	**95% CI**		**P-Value**
	No.	***(%)[4]***	***No.***	***(%)[4]***		***Lower***	***Upper***			***Lower***	***Upper***			***Lower***	***Upper***	
Vasectomy Ever-Use																
No	98	(64)	412	(55)	1.00				1.00				1.00			
Yes	54	(36)	332	(45)	0.67	0.46	0.96	0.039	0.82	0.54	1.23	0.325	0.83	0.55	1.24	0.358
Years of use																
Never	98	(66)	412	(56)	1.00				1.00				1.00			
<1 (0)	9	(6)	24	(3)	2.04	0.91	4.62	0.132	2.20	0.93	5.22	0.075	2.32	0.97	5.55	0.059
1-5	9	(6)	44	(6)	0.82	0.38	1.75	0.739	0.97	0.44	2.15	0.936	1.00	0.45	2.22	0.994
6-10	8	(5)	56	(8)	0.58	0.27	1.22	0.185	0.67	0.30	1.51	0.338	0.69	0.31	1.55	0.370
11-15	4	(3)	55	(7)	0.27	0.10	0.79	0.019	0.38	0.13	1.12	0.080	0.39	0.13	1.14	0.085
16-20	7	(5)	52	(7)	0.52	0.23	1.18	0.149	0.63	0.27	1.49	0.296	0.62	0.26	1.47	0.280
21-25	7	(5)	45	(6)	0.72	0.31	1.69	0.584	1.03	0.42	2.51	0.944	1.03	0.42	2.50	0.953
>25 (26-40)	7	(5)	51	(7)	0.67	0.29	1.55	0.454	0.58	0.23	1.47	0.251	0.56	0.22	1.43	0.222
Trend Test – per year					*0.969*	*0.94*	*1.000*	*0.051*	*0.971*	*0.939*	*1.004*	*0.080*	*0.969*	*0.936*	*1.002*	*0.065*

[1]Adjusted for age in five-year groups

[2]Adjusted for age in 5-year groups; PMH (ever-use); OCs (ever-use); Parity (grouped into 1, 2, 3, and ≥4); history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (grouped into: ≤25, 26-30, 31-35, and >35); history of infertility.

[3]Additionally adjusted for ever-use of DMPA, and ever-use of IUD.

[4]Percentages are of total stated

10.3.3 ANALYSIS WITH WOMEN WHO HAVE USED NEITHER HORMONAL CONTRACEPTIVES (DMPA/OCS) NOR HAD A VASECTOMISED PARTNER AS THE REFERENCE GROUP

There were 30 cases and 54 controls who had never used OCs or DMPA and never had a vasectomised partner. When these women were used as the reference group (Table 10.2), a stronger inverse association was observed (OR = 0.29; 95% CI = 0.17-0.50). Longer duration of use was more strongly inversely associated with risk. The strongest inverse association was observed in women with history of reliance on vasectomy for 11-15 years (OR = 0.10; 95% CI = 0.03-0.34), whereas no association was seen in women with history of reliance on vasectomy for <1 year (OR = 0.89; 95% CI = 0.35-2.23 for ever- versus never-use). In multivariate analysis, the ORs were attenuated (OR = 0.44; 95% CI = 0.11–1.76), although an overlapping of confidence intervals with those of age-adjusted analysis was observed.

Table 10.2: Risk of ovarian cancer in women with a history of ever having a vasectomised partner compared with those with neither a history of having a vasectomised partner nor use of oral contraceptives or DMPA

	Cases		**Controls**		**OR[1]**	**95% CI**		**P-Value**	**OR[2]**	**95% CI**		**P-Value**
	No.	***(%)[3]***	***No.***	***(%)[3]***		***Lower***	***Upper***			***Lower***	***Upper***	
Vasectomy Use												
Ever	30	(36)	54	(14)	1.00				1.00			
Never	54	(64)	332	(86)	0.29	0.17	0.50	<.001	0.44	0.11	1.76	0.244
Years of use												
Never	30	(37)	54	(14)	1.00				1.00			
<1 (0)	9	(11)	24	(6)	0.89	0.35	2.23	0.983	0.82	0.17	3.94	0.805
1-5	9	(11)	44	(12)	0.32	0.13	0.78	0.017	0.43	0.09	2.09	0.295
6-10	8	(10)	56	(15)	0.30	0.13	0.67	0.003	0.26	0.05	1.34	0.108
11-15	4	(5)	55	(14)	0.10	0.03	0.34	<0.001	0.16	0.03	1.01	0.051
16-20	7	(9)	52	(14)	0.25	0.10	0.63	0.004	0.29	0.06	1.40	0.122
21-25	7	(9)	45	(12)	0.33	0.12	0.89	0.043	0.41	0.08	2.23	0.303
>25 (26-40)	7	(9)	51	(13)	0.32	0.12	0.86	0.036	0.23	0.04	1.28	0.093
Trend Test – per year									*0.971*	*0.939*	*1.004*	*0.080*

[1]Adjusted for age in five-year groups

[2]Adjusted for age in 5-year groups; PMH (ever-use); OCs (ever-use); Parity (grouped into 1, 2, 3, and ≥4); history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (grouped into: ≤25, 26-30, 31-35, and >35); history of infertility.

[3]Percentages are of total stated

10.3.4 ANALYSIS RESTRICTED TO EVER-USERS OF HORMONAL CONTRACEPTIVES

The association between partner vasectomy and the risk of ovarian cancer was also assessed confined to women who had ever used OCs or DMPA (Table 10.3). Ever-use of vasectomy was associated with an OR of 0.81 (95% CI = 0.54-1.21). No duration-response relationship was observed. Similar observations were made in multivariate analysis.

Table 10.3: Risk of ovarian cancer in women with a history of having a vasectomised partner compared with those with no history of having a vasectomised partner in women who have ever used hormonal contraceptives (OCs or DMPA)

	Cases	Controls	OR[1]	95% CI		P-	OR[2]	95% CI		P-
	No. (%)[3]	*No. (%)*[3]		*Lower*	*Upper*	Value		*Lower*	*Upper*	Value
Vasectomy										
Never	68 (58)	358 (53)	1.00				1.00			
Ever	50 (42)	316 (47)	0.81	0.54	1.21	0.355	0.84	0.54	1.28	0.410
Years of Use										
Never	68 (59)	358 (53)	1.00				1.00			
<1 (0)	8 (7)	22 (3)	2.57	1.07	6.15	0.058	2.53	1.02	6.29	0.046
1-5	9 (8)	40 (6)	1.17	0.54	2.56	0.849	1.19	0.53	2.69	0.676
6-10	6 (5)	55 (8)	0.55	0.24	1.30	0.219	0.52	0.21	1.29	0.157
11-15	4 (3)	55 (8)	0.35	0.12	1.01	0.069	0.37	0.13	1.09	0.072
16-20	7 (6)	47 (7)	0.70	0.30	1.60	0.496	0.77	0.32	1.84	0.551
21-25	7 (6)	43 (6)	0.92	0.39	2.17	0.989	1.05	0.43	2.58	0.915
>25 (26-40)	6 (5)	49 (7)	0.73	0.29	1.81	0.638	0.61	0.24	1.59	0.314
Trend Test			*1.000*	*1.000*	*1.001*	*0.234*	*0.975*	*0.942*	*1.009*	*0.145*
Never used hormonal contraceptives (DMPA/OCs)										
Vasectomy Use										
Ever	30 (88)	54 (77)	1.00				1.00			
Never	4 (12)	16 (23)	0.53	0.17	1.71	0.407	0.52	0.11	2.53	0.244

[1]Adjusted for age in five-year groups
[2]Adjusted for age in 5-year groups; PMH (ever-use); OCs (ever-use); Parity (grouped into 1, 2, 3, and ≥4); history of ovarian, breast, endometrial, or colorectal cancer in a first-degree relative; age at last delivery (grouped into: ≤25, 26-30, 31-35, and >35); history of infertility.
[3]Percentages are of total stated

10.3.5 ANALYSIS RESTRICTED TO NEVER-USERS OF HORMONAL CONTRACEPTIVES

There were 4 cases and 16 controls with a history of union with a vasectomised partner, who had never used OCs or DMPA. When compared to women who had never used hormonal contraceptives and had never had a vasectomised partner (Table 10.3, bottom panel), ever-use of vasectomy was associated with an OR of 0.53 (95% CI = 0.17-1.71). No change was observed in multivariate analysis (OR = 0.52; 95% CI = 0.11–2.53).

10.3.6 RISK OF OVARIAN CANCER ASSOCIATED WITH EVER-USE OF VASECTOMY BY HISTOLOGIC TYPE

Risks of serous and non-serous tumours were assessed in women with history of union with a vasectomised partner, adjusted for age in 5-year groups (Table 10.4). Ever having a vasectomised partner was inversely associated with non-serous tumours (OR = 0.41; 95% CI = 0.22-0.79), but was not associated with serous tumours (OR = 0.83; 95% CI = 0.53-1.31).

Associations with mucinous and non-mucinous tumours were also assessed, adjusted for age (categorised into <60 years and ≥60 years due to the small number of cases with mucinous tumours) (Table 10.4). There were no statistically significant associations with mucinous or

non-mucinous tumours (OR = 0.53; 95% CI = 0.14-2.07, and OR = 0.69; 95% CI = 0.47-1.01, respectively).

Table 10.4: Risk of ovarian cancer associated with ever having had a vasectomised partner by histological type

	Cases *No. (%)*[1]	Controls *No. (%)*[1]	OR	95% CI *Lower Upper*	P-value
	Serous[2]				
Vasectomy Use					
Never	53 (59)	412 (55)	1.00		
Ever	37 (41)	332 (45)	0.83	0.53 1.31	0.495
	Non-serous[2]				
Vasectomy Use					
Never	39 (75)	412 (55)	1.00		
Ever	13 (25)	332 (45)	0.41	0.22 0.79	0.009
	Mucinous[3]				
Vasectomy Use					
Never	7 (70)	412 (55)	1.00		
Ever	3 (30)	332 (45)	0.53	0.14 2.07	0.546
	Non-Mucinous[3]				
Vasectomy Use					
Never	85 (64)	412 (55)	1.00		
Ever	47 (36)	332 (45)	0.69	0.47 1.01	0.073

[1]Percentages are of total stated
[2]ORs adjusted for age in five-year groups
[3]ORs adjusted for age (categorised into <60 and ≥60 years).

10.4 DISCUSSION

Discussion of previous studies assessing the association between partner vasectomy and the risk of ovarian cancer, and possible biologic mechanisms for this association have been provided in Chapter 4 (sections 4.4.5 and 4.5.5). Herein, a comparison of the findings of this study with those of previous studies and a discussion of the strengths and limitations of this study will be covered (more detailed discussion of the strengths and limitations of this study is also provided in Chapter 11- section 11.2).

In this study, history of union with a vasectomised partner was associated with a statistically significant lower risk in age-adjusted analysis, but this was attenuated in multivariate analysis (from 33% lower in age-adjusted to 18% in multivariate analysis). An inverse association was observed between duration of reliance on vasectomy and the risk of ovarian cancer. A stronger inverse association was observed when women who had never used hormonal contraceptives (OCs/DMPA) were used as the reference group.

Consistent with the findings of this study, in two previous studies assessing the association between partner vasectomy and ovarian cancer, inverse associations were reported [7, 14]. Ness et al. reported statistically significant findings comparable to those of the current study. Ever- compared to never-use of vasectomy was associated with an OR of 0.77 (95% CI = 0.61–0.97). In addition, consistent with our findings, when analysis was done with women who had never used any type of contraception as the reference group, a greater inverse association was observed (OR = 0.48; 95% CI = 0.31–0.79 - adjusted for age, parity, race, infertility, and history of ovarian cancer) [14]. No previous study has assessed the association between duration of use of vasectomy for contraception and ovarian cancer.

The findings of this study and those of previous studies suggest an inverse association between partner vasectomy and ovarian cancer. The probable mechanisms of protection have already been discussed in Chapter 4 (section 4.5.5.4). Lower risk may be attributed, in part, to use of other contraceptives, particularly oral contraceptives, which have been shown to be inversely associated with ovarian cancer, although adjustment for use of other contraceptives did not change the direction of effect. In addition, women whose partners undergo vasectomy are more likely to be parous. However, in this study, when analysis was restricted to women who had never used OCs or DMPA, odds ratios below 1.00 were found, even after adjustment for parity. Previous studies have also reported lower risk after adjustment for parity [7, 14].

The strengths of this study, with respect to vasectomy, are its population-based design, and a high-prevalence of vasectomy (44%) in the study population. This is also one of few studies to assess the association between vasectomy and ovarian cancer and the first study to assess the association with duration of use of vasectomy as a contraceptive method. However, there are limitations that need to be considered. First, information regarding vasectomy was sought from women rather than their partners. It is possible that some women were not aware of their partner's vasectomy status, which would have resulted in under-reporting, but this would have biased the results towards the null. Second, this being a case-control study reliant on participants' memory of past exposure, there is the risk of information bias; however, participants may forget the timing of vasectomy but are much less likely not to know their partner's vasectomy status.

Findings regarding the association between partner vasectomy and ovarian cancer are inconclusive but encouraging; further research regarding this and changes in semen

composition post-vasectomy are needed. These will aid in better understanding of possible causes of ovarian cancer and may be important in the development of preventive strategies. If the inverse association between vasectomy and ovarian cancer is a valid finding and – causal, this would establish an important non-contraceptive benefit of vasectomy.

CHAPTER 11: DISCUSSION

11.1 SUMMARY OF FINDINGS

In this study, ever-use of oral contraceptives was inversely associated with the risk of ovarian cancer (OR = 0.35; 95% CI = 0.22-0.55). A trend toward lower risk with longer duration of use and higher risk with longer time since last use was observed. This is consistent with the findings of other studies [7, 63, 83, 240]. These findings fulfil Bradford Hill's causal criteria of dose-response relationship, consistency, and strength of association. In addition, several mechanisms explaining the inverse association between ovarian cancer and use of oral contraceptives have been proposed (see Chapter 4 – section 4.5.2), which would meet Bradford Hill's criteria of biological plausibility and coherence [144]. The consistency of the findings of this study with those of other studies, particularly with regard to parity, breastfeeding, and use of oral contraceptives (see Chapter 7) which show well-established inverse associations with ovarian cancer, improves confidence in the validity of the findings of this study.

History of having a vasectomised partner was inversely associated with ovarian cancer. A suggestive trend towards lower risk with longer duration of use was observed (P-trend = 0.051). Inverse association between partner vasectomy and ovarian cancer have also been reported in previous studies [7, 14]. These findings fulfil Bradford Hill's criteria of consistency and dose-response relationship. However, there is a paucity of knowledge about any possible mechanisms of effect of vasectomy on the risk of ovarian cancer.

In this study, ever-use of DMPA was associated with an OR of 0.70 (95% CI = 0.38-1.30) for ovarian cancer. Other studies have reported lower risk of ovarian cancer in ever-users of DMPA [12, 13]. No statistically significant trend of lower risk with longer duration of use of DMPA or higher risk with longer time since last use was observed in this study. A duration-response relationship has previously been reported in one study [13]. Differences in the direction of association of DMPA with histological types (serous and mucinous tumours) were observed in both this study and the WHO Collaborative Study of Neoplasia and Steroid Contraceptives [10]. These observations are similar to those for oral contraceptives and ovarian cancer, and fulfil Bradford Hill's criteria of consistency and specificity. Proposed mechanisms of effect of DMPA on the pathogenesis of ovarian cancer (discussed in Chapter 4 – section 4.5.3), are consistent with an inverse association.

Ever-use of IUDs was not associated with ovarian cancer. However, longer duration of use was associated with higher risk of ovarian cancer (P-trend = 0.030) and longer time since last use was inversely associated with ovarian cancer (P-trend = 0.032). Previous studies have observed lower and higher risks in ever-users of IUDs. This inconsistency in findings does not support an association between ovarian cancer and the use of IUDs. However, the dose-duration response observed in both this study and that of Ness et al. [14], and the differences in risk by histologic type, which were observed by Tworoger et al. [7], support a possible causal association.

This study, interpreted in the context of Bradford Hill's causal criteria [144], suggests that use of DMPA and partner vasectomy may lower the risk of ovarian cancer, whereas use of IUDs (particularly copper-bearing IUDs) may increase the risk of ovarian cancer. However, most of the findings in this study were not statistically significant, which may be attributed to low power. Despite this, the findings of this study add to the body of knowledge on the association between use of contraceptives and the risk of ovarian cancer. In addition, when the findings of this study are compared with the findings of other studies and consistency in direction of effect is demonstrated, it may suggest causal associations. However, as a stand-alone study the findings may be attributed to chance and should therefore be interpreted with caution.

Biases and errors that may have influenced the findings of this study are discussed in section 11.2.

11.2 STRENGTHS AND LIMITATIONS OF THE STUDY

In this section, strengths and limitations of the study will be discussed. Where there are possible biases, the steps that were taken to minimise these will also be discussed.

11.2.1 STRENGTHS OF THE STUDY

The strengths of this study are its nationwide population-based design and that important predictors of ovarian cancer were collected; we were therefore, able to control for confounding. In addition, it is one of few studies done to assess the association between risk of ovarian cancer and use of DMPA, IUDs, and vasectomy. Furthermore, New Zealand has a high prevalence of ever-use of vasectomy (44%) and therefore provides an ideal population in which to investigate a possible association between vasectomy and ovarian cancer.

11.2.2 LIMITATIONS OF THE STUDY

The following were identified as limitations of this study:

11.2.2.1 Low power

This study had low power, which was mainly due to the low response proportion among controls; this may have hampered the detection of a true association between use of contraceptives (DMPA, IUDs, and partner vasectomy) and the risk of ovarian cancer. New Zealand's relatively small population meant that recruiting an adequate number of cases was a challenge. In addition, there was the possibility of research fatigue because, for instance, potential participants were already involved in other studies and as a result did not take part in this study (some eligible women stated that they were already involved in other studies).

11.2.2.2 Selection bias

In a case-control study, there is the risk of selection bias. This can be introduced by inappropriate selection of cases and controls or low levels of participation or both. In order to avoid this, other than the requirement of a histologic diagnosis of ovarian cancer, the controls fulfilled all the eligibility criteria defined for the cases (described in Chapter 5). To ensure that the controls represented the population from which the cases were drawn, and because controls were randomly selected from the electoral roll, all participants, including cases, had to be listed on the electoral roll.

This study was limited by the low response proportion (48% for controls and 74% for cases). It was therefore possible that respondents differed systematically from non-respondents. Lack of detailed information on non-participants precluded the assessment of whether participants differed from non-participants in exposures of interest. However, the age distribution of respondents was not different from the expected age of all potential participants contacted for both the case and control series (Table 5.2). In addition, cases who participated did not differ from those who did not by age, histological type of tumour, or stage of disease (Tables 5.2, 5.3, and 5.4). When response among controls by geographic region was assessed, a higher response proportion was noted in the South Island than in the North Island (Table 5.5). However, when only participants with whom contact was achieved were considered (i.e., excluding those who could not be located), participation was equal across regions (Table 5.6). This difference may be explained by the higher population mobility in the North Island: the Auckland region has the highest proportion of people who change residences between censuses, with some areas having only 10% of the population living at the same address between the 2001 and 2006 censuses [296]. Similar studies in New Zealand in the 1980s [18] and 1990s [288] had higher response proportions (84% and 85% respectively). In contrast to the current study, the inclusion criteria for those studies required participants to have

traceable telephone numbers. Household access to a landline telephone has decreased in New Zealand (from 96.3% in 2001 to 85.5% in 2013) accompanied by, and almost certainly explained by, a rise in the use of cell phones (from 74.2% in 2006 to 83.7 % in 2013) [304].

Response proportions in population-based case-control studies have been declining especially among controls [307, 308]. Response proportions equivalent to those of the current study are becoming more common [307]. For instance, in the Australian Ovarian Cancer Study, an Australian nationwide population-based case-control study, 47% of eligible controls took part in the study [78, 309].

In the study reported here, a self-administered postal questionnaire was used to collect information; this has the limitation that it can only be used in a literate population. This was not a major limitation because the literacy level in the New Zealand population is high. The Adult Literacy and Life Skills (ALL) survey of 2006 reported that 87% of New Zealanders have literacy levels of 2 and above, which was adequate for this study[5] [310]. The questions were framed in simple language for easier understanding. In addition, in order to improve participation, those who did not respond after 6 weeks from initial mail-out of the questionnaire, including no response to the second mail-out, were contacted and offered the option of completing the questionnaire by telephone interview; this provided an option for those participants who were not comfortable with a self-administered questionnaire. Among controls, 19% (158/837) of the respondents were interviewed by telephone, whereas among cases, 26% (40/152) of the respondents completed the questionnaire via telephone interviews, which increased the response proportion from 39.1% to 48.2% and from 54.6% to 74.1% respectively.

Another potential source of selection bias in this study was the exclusion of women who were unable to communicate in English. The ideal would have been to provide interpreters to the participants who could not communicate in English but, due to cost, this group was excluded. This would potentially introduce selection bias, but English being an official language and the most spoken language in New Zealand, the effect was minimal. In addition, participants were New Zealand citizens and permanent residents (as they were recruited from the electoral rolls), which further limited the number of non-English speaking potential participants. As

[5] ALL assigns participants to one of 5 cognitive levels; level 1 being the lowest and level 5 the highest. Level 2 includes tasks that demand the capacity to search a document and filter out some simple distracting information, achieve low-level inferences, and execute one- or two-step calculations and estimations.

had been expected, a low number; only 16 (0.8%) of potential control subjects did not meet this criterion.

Selection bias may under- or over-estimate risk associated with an exposure and limits generalizability of study findings. Assessing the extent to which selection bias has affected the findings in population-based case-control studies is usually difficult due to lack of exposure information of non-participants [308].

11.2.2.3 Recall bias

In a case-control study, participants provide estimates of past exposures. Accurate measures can be difficult to obtain and the degree of accuracy can differ between cases and controls, which can lead to information bias; this in turn may lead to under- or over-estimation of risk associated with the exposures [311]. To improve recall, participants were provided with a calendar of major life events to assist in recall and to record use of contraceptives. In order to ensure that there were no differences in recall between the cases and controls, all participants completed the same questionnaire. The participants were also unaware of the study hypothesis and their case or control status. The study hypothesis was concealed by using a different title from that of the study on the invitation letters, information sheets, consent forms and questionnaires. This is common practice in case-control studies and is supported by "the International Ethical Guidelines for Epidemiological Studies" [292].

In addition, the association between contraceptive use and the risk of ovarian cancer has not been widely publicised; therefore, this would not contribute to differential response between cases and controls regarding the use of contraceptives. Although women with ovarian cancer may have read widely about possible causal and protective factors, which could contribute to reporting bias, there was consistency between studies for many of the findings. Consistency between this study and other case-control studies (particularly with respect to OCs and ovarian cancer), suggests that bias did not substantially affect the findings (bias would not be expected to operate in exactly the same way in different studies carried out in different populations).

11.2.2.4 Survival bias

To avoid survival bias, only incident cases were included in the study. Despite this, women with ovarian cancer who took part in the study may have had less advanced disease than those who were eligible for the study but did not participate. This is because of the time taken from diagnosis to participation. The average duration from diagnosis to receipt of histological

reports by the researchers was 3.0 months (SD = 2.3), and the average time from diagnosis to participation was 5.1 months (SD = 2.4). Analysis of stage of disease was limited by the lack of information from participants' medical records and that the majority (140 out of 258 [54.3%]) of the histology reports did not indicate stage of disease. However, analysis of those who had information on stage of disease did not show evidence of any difference between participants and non-participants (Table 5.4). In addition, of all the cases received from the New Zealand Cancer Registry (NZCR), only 3.9% (10/258) had died before contact could be made and, of the six potential cases whose treating doctors advised us against contacting them, only two were due to advanced disease. Due to the risk of survival bias, the findings of this study may be applicable to women with less advanced disease only.

11.2.2.5 Misclassification of exposure

Early symptoms of ovarian cancer may have influenced the habits of case subjects and therefore influenced reported exposures. Information regarding change in exposure habits post-diagnosis was not collected. In addition, the study relied on self-reported exposures which were not verified by medical records. However, although it is possible that participants may forget patterns of use of a particular contraceptive (age of onset, duration of use and age at stopping use), they are less likely to forget the type of contraceptive used [14]. In addition, in previous New Zealand studies in which corroborative information was obtained from medical practitioners, information on contraceptive use provided by the participants was consistent with that in their medical records [18, 288]. There was also the risk of histologic misclassification due to inter-pathologist variation. This is because the study relied on histological classification by the NZCR (from histology forms) and the histological slides were not all reviewed by one pathologist. Assuming any misclassification was non-differential, this may have biased the results towards the null.

11.2.2.6 Other possible sources of confounding

The presence of confounders that were not accounted for cannot be ruled out in any observational study. In addition, the study did not test for genetic predisposition to ovarian cancer. To mitigate this, family and personal history of cancer was recorded in order to give insight into a possibility of genetic predisposition. In order to avoid preferential recall of family history of cancer by cases, only history of cancer in a first-degree relative was considered in determining the possible presence of familial predisposition to ovarian cancer.

Case-control studies have the potential for bias; the challenge is that although it may be easy to identify the potential sources of bias, and it may be possible to predict the direction of

effect of the bias, it is rarely possible to estimate the true impact that these biases may have had on the results. However, if conducted well, a case-control study can yield valid and informative results.

11.3 IMPLICATIONS OF THE FINDINGS

If the directions of association observed in this study are true:

- the inverse associations for both partner vasectomy and use of DMPA with the occurrence of ovarian cancer provide additional important non-contraceptive benefits of vasectomy and DMPA. This may provide alternative contraceptive options for women who wish to lower their risk of ovarian cancer but cannot use oral contraceptives.
- the inverse association between DMPA and ovarian cancer further supports the protective effect of progesterone. Furthermore, the stronger inverse association with older age at first use supports the clearing of malignant epithelial cells by progesterone, similar to what is seen with older age at last delivery.
- the positive association between use of IUDs and ovarian cancer lends further credence to the inflammatory hypothesis in ovarian carcinogenesis.
- constituents of semen that are eliminated or decreased post-vasectomy may be involved in promoting ovarian carcinogenesis. Establishment of their mechanisms of effect on ovarian carcinogenesis would support the already proposed pathogenic pathways and/or provide new knowledge on ovarian carcinogenesis.

11.4 CONCLUSIONS AND RECOMMENDATIONS

The findings of this study suggest that ovarian cancer may be inversely associated with partner vasectomy and DMPA and positively associated with IUDs. However, these findings are inconclusive. This is partly because of low power and, as a probable result, are associated with some statistically non-significant findings. In addition, the lack of a complete understanding of the pathogenesis of ovarian cancer and how these contraceptives affect ovarian carcinogenesis further limits definitive conclusions. Furthermore, there is lack of consistency across studies. Therefore, the following recommendations are made regarding future research endeavours:

- a study with enough power - or a collaborative analysis of existing studies – should be undertaken to assess the association between IUDs, long-acting progestogen-based

contraceptives, and partner vasectomy and the risk of ovarian cancer as well as differences in risk according to IUD type.

- further research should be undertaken to investigate changes in semen composition pre- and post-vasectomy and how these may affect ovarian carcinogenesis.
- unlike the position for colorectal and breast cancers, New Zealand is not among the highest risk countries for ovarian cancer. The high prevalence of use of oral contraceptives and vasectomy may have influenced this; however, this requires more examination. A study assessing the incidence trends of ovarian cancer in New Zealand in relation to use of oral contraceptives and vasectomy in comparison to other countries is therefore recommended.

The use of contraceptives is potentially under the control of an individual and thus modifiable. Knowledge of the effects of contraceptives on the health of users is important in assessing the risks and benefits of a contraceptive when choosing a method. Contraceptives are widely used and use may span months or years; therefore, even a small effect on the risk of ovarian cancer may have a great impact on the incidence of disease in the general population.

REFERENCES

1. Jemal, A., M.M. Centre, C. DeSantis, and E.M. Ward, *Global patterns of cancer incidence and mortality rates and trends.* Cancer Epidemiology, Biomarkers and Prevention, 2010. **19**(8): p. 1893-907.
2. Jemal, A., F. Bray, M.M. Center, J. Ferlay, E. Ward, and D. Forman, *Global cancer statistics.* CA: A Cancer Journal for Clinicians, 2011. **61**(2): p. 69-90.
3. Ministry of Health, *Cancer: new registrations and deaths 2012*. 2015, Ministry of Health: Wellington, New Zealand.
4. Ministry of Health, *Cancer patient survival covering the period 1994 to 2007*. 2010, Ministry of Health: Wellington, New Zealand:
5. Ness, R.B., J.A. Grisso, R. Vergona, J. Klapper, M. Morgan, and J.E. Wheeler, *Oral contraceptives, other methods of contraception, and risk reduction for ovarian cancer.* Epidemiology, 2001. **12**(3): p. 307-12.
6. Beral, V., R. Doll, C. Hermon, R. Peto, and G. Reeves, *Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls.* The Lancet, 2008. **371**(9609): p. 303-14.
7. Tworoger, S., K. Fairfield, G. Colditz, B. Rosner, and S. Hankinson, *Association of oral contraceptive use, other contraceptive methods, and infertility with ovarian cancer risk.* American Journal of Epidemiology, 2007. **166**(8): p. 894-901.
8. Webb, P., D. Gertig, and D. Hunter, Ovarian cancer, in textbook of cancer epidemiology. Eds, H.O. Adami, D. Hunter, and D. Trichopoulos. 2008, Oxford University Press, doi:10.1093/acprof:oso/9780195311174.003.0019
9. Iodice, S., M. Barile, N. Rotmensz, I. Feroce, B. Bonanni, and P. Radice, *Oral contraceptive use and breast or ovarian cancer risk in BRCA 1/2 carriers: a meta-analysis.* European Journal of Cancer, 2010. **46**(12): p. 2275-84.
10. Stanford, J.L. and D.B. Thomas, *Depot-medroxyprogesterone acetate (DMPA) and risk of epithelial ovarian cancer. The WHO Collaborative Study of Neoplasia and Steroid Contraceptives.* International Journal of Cancer, 1991. **49**: p. 191-5.
11. Shu, X.O., L.A. Brinton, Y.T. Gao, and J.M. Yuan, *Population-based case-control study of ovarian cancer in Shanghai.* Cancer Research, 1989. **49**(13): p. 3670-3674.
12. Urban, M., E. Banks, S. Egger, K. Canfell, D. O'Connell, V. Beral, et al., *Injectable and oral contraceptive use and cancers of the breast, cervix, ovary, and endometrium*

in black South African women: case-control study. PLoS Medicine, 2012. **9**(3): p. e1001182.

13. Wilailak, S., C. Vipupinyo, V. Suraseranivong, Chotivanich K, C. Kietpeerakool, and t.Y. Tanapa, *Depot medroxyprogesterone acetateand epithelial ovarian cancer: a multicentre case-control study.* BJOG: An International Journal of Obstetrics & Gynaecology, 2012. **119**(6): p. 672-7.
14. Ness, R.B., R.C. Dodge, R.P. Edwards, J.A. Baker, and K.B. Moysich, *Contraception methods, beyond oral contraceptives and tubal ligation, and risk of ovarian cancer.* Annals of Epidemiology, 2011. **21**(3): p. 188-196.
15. Hankinson, S.E., D.J. Hunter, G.A. Colditz, W.C. Willett, M.J. Stampfer, B. Rosner, et al., *Tubal ligation, hysterectomy, and risk of ovarian cancer: a prospective study.* Journal of the American Medical Association, 1993. **270**(23): p. 2813-2818.
16. Faculty of Sexual and Reproductive Healthcare, *Combined hormonal contraception*. 2012, London: Faculty of Sexual and Reproductive Healthcare.
17. World Health Organization, *Medical eligibility criteria for contraceptive use*. 2010, WHO: Geneva.
18. Paul, C., D.C. Skegg, J. Smeijers, and G.F. Spears, *Contraceptive practice in New Zealand.* The New Zealand Medical Journal, 1988. **101**(859): p. 809.
19. Chen, V.W., B. Ruiz, J.L. Killeen, T.R. Coté, X.C. Wu, C.N. Correa, et al., *Pathology and classification of ovarian tumors.* Cancer, 2003. **97**(S10): p. 2631-2642.
20. Hall, J.E., *Guyton and Hall textbook of medical physiology*. 12th ed. 2011, Philadelphia, PA: Saunders.
21. Kovacs, W.J. and S.R. Ojeda, *Textbook of endocrine physiology*. 2011, Oxford University Press: Oxford.
22. Moore, K.L. and T.V.N. Persaud, *The developing human: clinically oriented embryology*. 7 ed. 2003, Philadelphia, PA: Saunders.
23. DeCherney, A.H., L. Nathan, T.M. Goodwin, and N. Laufer, *Current diagnosis & treatment obstetrics & gynecology*. 10th ed, ed. Anne M.Sydor and K. Edmonson. 2007, New York: McGraw-Hill.
24. Kaku, T., S. Ogawa, Y. Kawano, Y. Ohishi, H. Kobayashi, T. Hirakawa, et al., *Histological classification of ovarian cancer.* Medical Electron Microscopy, 2003. **36**(1): p. 9-17.
25. Jelovac, D. and D.K. Armstrong, *Recent progress in the diagnosis and treatment of ovarian cancer.* CA Cancer Journal for Clinicians, 2011. **61**(3): p. 183-203.

26. Soegaard, M., A. Jensen, E. Hogdall, L. Christensen, C. Hogdall, J. Blaakaer, et al., *Different risk factor profiles for mucinous and nonmucinous ovarian cancer: results from the Danish MALOVA study.* Cancer Epidemiology, Biomarkers and Prevention, 2007. **16**(6): p. 1160-6.
27. Dursun, P., M. Gultekin, K. Yuce, and A. Ayhan, *Lower expression of cyclooxygenase-2: is it associated with the development of borderline ovarian tumors?* Medical Hypotheses, 2005. **64**(2): p. 273-8.
28. Riman, T., P.W. Dickman, S. Nilsson, N. Correia, H. Nordlinder, C.M. Magnusson, et al., *Risk factors for epithelial borderline ovarian tumors: results of a Swedish case - control study.* Gynecologic Oncology, 2001. **83**(3): p. 575-585.
29. Chen, T., H.M. Surcel, E. Lundin, M. Kaasila, H.A. Lakso, H. Schock, et al., *Circulating sex steroids during pregnancy and maternal risk of non-epithelial ovarian cancer.* Cancer Epidemiology Biomarkers and Prevention, 2011. **20**(2): p. 324-336.
30. Merritt, M.A., M. De Pari, A.F. Vitonis, L.J. Titus, D.W. Cramer, and K.L. Terry, *Reproductive characteristics in relation to ovarian cancer risk by histologic pathways.* Human Reproduction, 2013. **28**(5): p. 1406-1417.
31. Poole, E.M., M.A. Merritt, S.J. Jordan, H.P. Yang, S.E. Hankinson, Y. Park, et al., *Hormonal and reproductive risk factors for epithelial ovarian cancer by tumor aggressiveness.* Cancer Epidemiology Biomarkers and Prevention, 2013. **22**(3): p. 429-436.
32. Gadducci, A., C. Sergiampietri, and R. Tana, *Alternatives to risk-reducing surgery for ovarian cancer.* Annals of Oncology, 2013. **24**(suppl 8): p. viii47-viii53.
33. Brunsvold, A.N., S.-F. Wung, and C.J. Merkle, *BRCA1 genetic mutation and its link to ovarian cancer: implications for advanced practice nurses.* Journal of the American Academy of Nurse Practitioners, 2005. **17**(12): p. 518-26.
34. Shimizu, Y., S. Kamoi, S. Amada, F. Akiyama, and S.G. Silverberg, *Toward the development of a universal grading system for ovarian epithelial carcinoma.* Cancer, 1998. **82**(5): p. 893-901.
35. Silverberg, S.G., *Histopathologic grading of ovarian carcinoma: a review and proposal.* International Journal of Gynecological Pathology, 2000. **19**(1): p. 7-15.
36. Argento, M., P. Hoffman, and A.-S. Gauchez, *Ovarian cancer detection and treatment: current situation and future prospects.* Anticancer Research, 2008. **28**(5B): p. 3135-8.

37. Mourits, M.J. and G.H. de Bock, *Managing hereditary ovarian cancer.* Maturitas, 2009. **64**(3): p. 172-176.
38. Lurie, G., L.R. Wilkens, P.J. Thompson, R.K. Matsuno, M.E. Carney, and M.T. Goodman, *Symptom presentation in invasive ovarian carcinoma by tumor histological type and grade in a multiethnic population: a case analysis.* Gynecologic Oncology, 2010. **119**(2): p. 278-84.
39. National Health Committee, *Screening to improve health in New Zealand: criteria to assess screening programmes*. 2003, National Advisory Committee on Health and Disability: Wellington.
40. Wilson, J.M.G. and G. Jungner, *Principles and practice of screening for disease.* World Health Organization, 1968(34).
41. Drerup, J.M., Y. Liu, A.S. Padron, K. Murthy, V. Hurez, B. Zhang, et al., *Immunotherapy for ovarian cancer.* Current Treatment Options in Oncology, 2015. **16**(1): p. 1-20.
42. Mantia-Smaldone, G.M., B. Corr, and C.S. Chu, *Immunotherapy in ovarian cancer.* Human Vaccines & Immunotherapeutics, 2012. **8**(9): p. 1179-1191.
43. Kalloger, S.E., M. Kobel, S. Leung, E. Mehl, D.X. Gao, K.M. Marcon, et al., *Calculator for ovarian carcinoma subtype prediction.* Modern Pathology, 2011. **24**(4): p. 512-521.
44. Kobel, M., S.E. Kalloger, N. Boyd, S. McKinney, E. Mehl, C. Palmer, et al., *Ovarian carcinoma subtypes are different diseases: implications for biomarker studies.* PLoS Medicine, 2008. **5**(12): p. 1749-1760.
45. Schorge, J.O., J.I. Schaffer, L.M. Halvorson, B.L. Hoffman, K.D. Bradshaw, and F.G. Cunnungham, *Williams gynecology*. 2008, New York: McGraw-Hill.
46. Rosner, B.A., G.A. Colditz, P.M. Webb, and S.E. Hankinson, *Mathematical models of ovarian cancer incidence.* Epidemiology, 2005. **16**(4): p. 508-15.
47. Folkins, A.K., E.A. Jarboe, M.H. Roh, and C.P. Crum, *Precursors to pelvic serous carcinoma and their clinical implications.* Gynecologic Oncology, 2009. **113**(3): p. 391-396.
48. Kurman, R.J. and I.M. Shih, *Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer-shifting the paradigm.* Human Pathology, 2011. **42**(7): p. 918-931.

49. Nezhat, F.R., R. Apostol, C. Nezhat, and T. Pejovic, *New insights in the pathophysiology of ovarian cancer and implications for screening and prevention.* American Journal of Obstetrics and Gynecology, 2015. **213**(3): p. 262-267.
50. Levanon, K., C. Crum, and R. Drapkin, *New insights into the pathogenesis of serous ovarian cancer and its clinical impact.* Journal of Clinical Oncology, 2008. **26**(32): p. 5284-5293.
51. Seidman, J.D., P. Zhao, and A. Yemelyanova, *"Primary peritoneal" high-grade serous carcinoma is very likely metastatic from serous tubal intraepithelial carcinoma: assessing the new paradigm of ovarian and pelvic serous carcinogenesis and its implications for screening for ovarian cancer.* Gynecologic Oncology, 2011. **120**(3): p. 470-473.
52. Kurman, R.J. and I.M. Shih, *The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory.* American Journal of Surgical Pathology, 2010. **34**(3): p. 433-443.
53. Risch, H.A., *Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone.* Journal of the National Cancer Institute, 1998. **90**(23): p. 1774-1786.
54. Karst, A.M. and R. Drapkin, *Ovarian cancer pathogenesis: a model in evolution.* Journal of Oncology, 2010. **2010**: p. 932371.
55. Fleming, J.S., C.R. Beaugié, I. Haviv, G. Chenevix-Trench, and O.L. Tan, *Incessant ovulation, inflammation and epithelial ovarian carcinogenesis: revisiting old hypotheses.* Molecular and Cellular Endocrinology, 2006. **247**(1–2): p. 4-21.
56. Dubeau, L., *The cell of origin of ovarian epithelial tumours.* Lancet Oncology, 2008. **9**(12): p. 1191-1197.
57. Lee, Y., F. Medeiros, D. Kindelberger, M.J. Callahan, M.G. Muto, and C.P. Crum, *Advances in the recognition of tubal intraepithelial carcininoma; applications to cancer screening and the pathogenesis of ovarian cancer.* Advances in Anatomic Pathology, 2006. **13**(Number 1): p. 1-7.
58. Hesketh, R., *Introduction to cancer biology*. 2013, New York: Cambridge University Press.
59. Sieh, W., S. Salvador, V. McGuire, R.P. Weber, K.L. Terry, M.A. Rossing, et al., *Tubal ligation and risk of ovarian cancer subtypes: a pooled analysis of case-control studies.* International Journal of Epidemiology, 2013. **42**(2): p. 579-589.

60. Li, J., N. Abushahin, S.J. Pang, L. Xiang, S.K. Chambers, O. Fadare, et al., *Tubal origin of 'ovarian' low-grade serous carcinoma.* Modern Pathology, 2011. **24**(11): p. 1488-1499.
61. Vanderhyden, B.C., *Loss of ovarian function and the risk of ovarian cancer.* Cell and Tissue Research, 2005. **322**(1): p. 117-124.
62. Tsilidis, K.K., N.E. Allen, T.J. Key, L. Dossus, A. Lukanova, K. Bakken, et al., *Oral contraceptive use and reproductive factors and risk of ovarian cancer in the European Prospective Investigation into Cancer and Nutrition.* British Journal of Cancer, 2011. **105**(9): p. 1436-42.
63. Pasalich, M., D. Su, C.W. Binns, and A.H. Lee, *Reproductive factors for ovarian cancer in southern Chinese women.* Journal of Gynecologic Oncology, 2013. **24**(2): p. 135-40.
64. Milne, R., A. Osorio, T. Ramón Y Cajal, M. Baiget, A. Lasa, E. Diaz-rubio, et al., *Parity and the risk of breast and ovarian cancer in BRCA1 and BRCA2 mutation carriers.* Breast Cancer Research and Treatment, 2010. **119**(1): p. 221.
65. Whiteman, D.C., M.F. Murphy, L.S. Cook, D.W. Cramer, P. Hartge, P.A. Marchbanks, et al., *Multiple births and risk of epithelial ovarian cancer.* Journal of the National Cancer Institute, 2000. **92**(14): p. 1172-7.
66. Gierach, G.L., F. Modugno, and R.B. Ness, *Gender of offspring and maternal ovarian cancer risk.* Gynecologic Oncology, 2006. **101**(3): p. 476-80.
67. Jordan, S.J., A.C. Green, C.M. Nagle, C.M. Olsen, D.C. Whiteman, P.M. Webb, et al., B*eyond parity: association of ovarian cancer with length of gestation and offspring characteristics.* American Journal of Epidemiology, 2009. **170**(5): p. 607.
68. Cooper, G.S., J.M. Schildkraut, A.S. Whittemore, and P.A. Marchbanks, *Pregnancy recency and risk of ovarian cancer.* Cancer Causes & Control, 1999. **10**(5): p. 397-402.
69. Whiteman, D.C., V. Siskind, D.M. Purdie, and A.C. Green, *Timing of pregnancy and the risk of epithelial ovarian cancer.* Cancer Epidemiology Biomarkers and Prevention, 2003. **12**(1): p. 42-46.
70. Baik, I., M. Lambe, Q. Liu, L. Chie, S. Cnattingius, L.A. Mucci, et al., *Birth spacing and maternal risk of invasive epithelial ovarian cancer in a Swedish nationwide cohort.* Cancer Causes and Control, 2008. **19**(10): p. 1131-1137.

71. Chen, M.T., L.S. Cook, J.R. Daling, and N.S. Weiss, *Incomplete pregnancies and risk of ovarian cancer (Washington, United States).* Cancer Causes & Control, 1996. **7**(4): p. 415-20.
72. Gierach, G.L., F. Modugno, and R.B. Ness, *Relations of gestational length and timing and type of incomplete pregnancy to ovarian cancer risk.* American Journal of Epidemiology, 2005. **161**(5): p. 452-461.
73. Beral, V., *Long term effects of childbearing on health.* Journal of Epidemiology and Community Health, 1985. **39**(4): p. 343-6.
74. Melin, A., P. Sparén, and A. Bergqvist, *The risk of cancer and the role of parity among women with endometriosis.* Human Reproduction, 2007. **22**(11): p. 3021.
75. Mills, P.K., D.G. Riordan, and R.D. Cress, *Epithelial ovarian cancer risk by invasiveness and cell type in the Central Valley of California.* Gynecologic Oncology, 2004. **95**(1): p. 215-25.
76. Danforth, K.N., S.S. Tworoger, J.L. Hecht, B.A. Rosner, G.A. Colditz, and S.E. Hankinson, *Breastfeeding and risk of ovarian cancer in two prospective cohorts.* Cancer Causes & Control, 2007. **18**(5): p. 517-523.
77. Luan, N.-N., Q.-J. Wu, T.-T. Gong, E. Vogtmann, Y.-L. Wang, and B. Lin, *Breastfeeding and ovarian cancer risk: a meta-analysis of epidemiologic studies.* The American Journal of Clinical Nutrition, 2013: p. 1-12.
78. Jordan, S.J., V. Siskind, A.C. Green, D.C. Whiteman, and P.M. Webb, *Breastfeeding and risk of epithelial ovarian cancer.* Cancer Causes and Control, 2010. **21**(1): p. 109-116.
79. Chiaffarino, F., C. Pelucchi, E. Negri, F. Parazzini, S. Franceschi, R. Talamini, et al., *Breastfeeding and the risk of epithelial ovarian cancer in an Italian population.* Gynecologic Oncology, 2005. **98**(2): p. 304-308.
80. Tung, K., L.R. Wilkens, A.H. Wu, K. McDuffie, A.M.Y. Nomura, L.N. Kolonel, et al., *Effect of anovulation factors on pre- and postmenopausal ovarian cancer risk: revisiting the incessant ovulation hypothesis.* American Journal of Epidemiology, 2005. **161**(4): p. 321-329.
81. Fathalla, M.F., *Incessant ovulation—a factor in ovarian neoplasia?* The Lancet, 1971. **298**(7716): p. 163.
82. Purdie, D.M., C.J. Bain, V. Siskind, P.M. Webb, and A.C. Green, *Ovulation and risk of epithelial ovarian cancer.* International Journal of Cancer, 2003. **104**(2): p. 228-32.

83. Pelucchi, C., C. Galeone, R. Talamini, C. Bosetti, M. Montella, E. Negri, et al., *Lifetime ovulatory cycles and ovarian cancer risk in 2 Italian case-control studies.* American Journal of Obstetrics and Gynecology, 2007. **196**(1): p. 83.e1-7.
84. Moorman, P.G., J.M. Schildkraut, B. Calingaert, S. Halabi, M.F. Vine, and A. Berchuck, *Ovulation and ovarian cancer: a comparison of two methods for calculating lifetime ovulatory cycles (United States).* Cancer Causes & Control, 2002. **13**(9): p. 807-11.
85. Terry, K.L., L. Titus-Ernstoff, J.R. McKolanis, W.R. Welch, O.J. Finn, and D.W. Cramer, *Incessant ovulation, mucin 1 immunity, and risk for ovarian cancer.* Cancer Epidemiology, Biomarkers & Prevention, 2007. **16**(1): p. 30-5.
86. Cramer, D.W., L. Titus-Ernstoff, J.R. McKolanis, W.R. Welch, A.F. Vitonis, R.S. Berkowitz, et al., *Conditions associated with antibodies against the tumor-associated antigen MUC1 and their relationship to risk for ovarian cancer.* Cancer Epidemiology Biomarkers and Prevention, 2005. **14**(5): p. 1125-1131.
87. Ness, R.B., J.A. Grisso, C. Cottreau, J. Klapper, R. Vergona, J.E. Wheeler, et al., *Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer.* Epidemiology, 2000. **11**(2): p. 111-7.
88. Ness, R.B., D.W. Cramer, M.T. Goodman, S.K. Kjaer, K. Mallin, B.J. Mosgaard, et al., *Infertility, fertility drugs, and ovarian cancer: a pooled analysis of case-control studies.* American Journal of Epidemiology, 2002. **155**(3): p. 217-24.
89. Jensen, A., H. Sharif, J.H. Olsen, and S.K. Kjær, *Risk of breast cancer and gynecologic cancers in a large population of nearly 50,000 infertile Danish women.* American Journal of Epidemiology, 2008. **168**(1): p. 49.
90. Brinton, L.A., E.J. Lamb, K.S. Moghissi, B. Scoccia, M.D. Althuis, J.E. Mabie, et al., *Ovarian cancer risk after the use of ovulation-stimulating drugs.* Obstetrics and Gynecology, 2004. **103**(6): p. 1194-1203.
91. Rossing, M.A., J.R. Daling, N.S. Weiss, D.E. Moore, and S.G. Self, *Ovarian tumors in a cohort of infertile women.* New England Journal of Medicine, 1994. **331**(12): p. 771-776.
92. Kurta, M.L., K.B. Moysich, J.L. Weissfeld, A.O. Youk, C.H. Bunker, R.P. Edwards, et al., *Use of fertility drugs and risk of ovarian cancer: results from a U.S.-based case-control study.* Cancer Epidemiology Biomarkers and Prevention, 2012. **21**(8): p. 1282-1292.

93. Venn, A., L. Watson, F. Bruinsma, G. Giles, and D. Healy, *Risk of cancer after use of fertility drugs with in-vitro fertilisation.* The Lancet, 1999. **354**(9190): p. 1586-1590.
94. Modan, B., E. Ron, L. Lerner-Geva, T. Blumstein, J. Menczer, J. Rabinovici, et al., *Cancer incidence in a cohort of infertile women.* American Journal of Epidemiology, 1998. **147**(11 SUPPL.): p. 1038-1042.
95. Nieto, J.J., J. Crow, M. Sundaresan, N. Constantinovici, C.W. Perrett, A.B. MacLean, et al., *Ovarian epithelial dysplasia in relation to ovulation induction and nulliparity.* Gynecologic Oncology, 2001. **82**(2): p. 344-9.
96. Vlahos, N.F., K.P. Economopoulos, and S. Fotiou, *Endometriosis, in vitro fertilisation and the risk of gynaecological malignancies, including ovarian and breast cancer.* Best Practice & Research: Clinical Obstetrics & Gynaecology, 2010. **24**(1): p. 39-50.
97. Brinton, L., *Long-term effects of ovulation-stimulating drugs on cancer risk.* Reproductive Biomedicine Online, 2007. **15**(1): p. 38-44.
98. Langseth, H., S.E. Hankinson, J. Siemiatycki, and E. Weiderpass, *Perineal use of talc and risk of ovarian cancer.* Journal of Epidemiology & Community Health, 2008. **62**(4): p. 358-60.
99. Cramer, D.W., R.F. Liberman, L. Titus-Ernstoff, W.R. Welch, E.R. Greenberg, J.A. Baron, et al., *Genital talc exposure and risk of ovarian cancer.* International Journal of Cancer, 1999. **81**(3): p. 351-6.
100. Wu, A.H., C.L. Pearce, C.C. Tseng, C. Templeman, and M.C. Pike, *Markers of inflammation and risk of ovarian cancer in Los Angeles County.* International Journal of Cancer, 2009. **124**(6): p. 1409-1415.
101. Merritt, M.A., A.C. Green, C.M. Nagle, and P.M. Webb, *Talcum powder, chronic pelvic inflammation and NSAIDs in relation to risk of epithelial ovarian cancer.* International Journal of Cancer, 2008. **122**(1): p. 170-176.
102. Wong, C., R.E. Hempling, M.S. Piver, N. Natarajan, and C.J. Mettlin, *Perineal talc exposure and subsequent epithelial ovarian cancer: a case-control study.* Obstetrics & Gynecology, 1999. **93**(3): p. 372-6.
103. Cibula, D., M. Widschwendter, O. Májek, and L. Dusek, *Tubal ligation and the risk of ovarian cancer: review and meta-analysis.* Human Reproduction Update, 2011. **17**(1): p. 55-67.

104. Narod, S.A., P. Sun, P. Ghadirian, H. Lynch, C. Isaacs, J. Garber, et al., *Tubal ligation and risk of ovarian cancer in carriers of BRCA1 or BRCA2 mutations: a case-control study.* The Lancet, 2001. **357**(9267): p. 1467-70.

105. Ness, R.B., M.T. Goodman, C. Shen, and R.C. Brunham, *Serologic evidence of past infection with Chlamydia trachomatis, in relation to ovarian cancer.* The Journal of Infectious Diseases, 2003. **187**(7): p. 1147.

106. Risch, H.A. and G.R. Howe, *Pelvic inflammatory disease and the risk of epithelial ovarian cancer.* Cancer Epidemiology, Biomarkers & Prevention, 1995. **4**(5): p. 447-51.

107. Ness, R.B., C. Shen, D. Bass, C. Jackson, K. Moysich, R. Edwards, et al., *Chlamydia trachomatis serology in women with and without ovarian cancer.* Infectious Diseases in Obstetrics and Gynecology, 2008. **2008**.

108. Cramer, D.W., B.L. Harlow, L. Titus-Ernstoff, K. Bohlke, W.R. Welch, and E.R. Greenberg, *Over-the-counter analgesics and risk of ovarian cancer.* The Lancet, 1998. **351**(9096): p. 104-7.

109. Wernli, K.J., P.A. Newcomb, J.M. Hampton, A. Trentham-Dietz, and K.M. Egan, *Inverse association of NSAID use and ovarian cancer in relation to oral contraceptive use and parity.* British Journal of Cancer, 2008. **98**(11): p. 1781-3.

110. Schildkraut, J.M., P.G. Moorman, S. Halabi, B. Calingaert, J.R. Marks, and A. Berchuck, *Analgesic drug use and risk of ovarian cancer.* Epidemiology, 2006. **17**(1): p. 104-107.

111. Hannibal, C.G., M.A. Rossing, K.G. Wicklund, and K.L. Cushing-Haugen, *Analgesic drug use and risk of epithelial ovarian cancer.* American Journal of Epidemiology, 2008. **167**(12): p. 1430.

112. Fairfield, K.M., D.J. Hunter, C.S. Fuchs, G.A. Colditz, and S.E. Hankinson, *Aspirin, other NSAIDs, and ovarian cancer risk (United States).* Cancer Causes & Control, 2002. **13**(6): p. 535-42.

113. Rosenberg, L., J.R. Palmer, R.S. Rao, P.F. Coogan, B.L. Strom, A.G. Zauber, et al., *A case-control study of analgesic use and ovarian cancer.* Cancer Epidemiology Biomarkers & Prevention, 2000. **9**(9): p. 933-937.

114. Akhmedkhanov, A., P. Toniolo, A. Zeleniuch-Jacquotte, I. Kato, K.L. Koenig, and R.E. Shore, *Aspirin and epithelial ovarian cancer.* Preventive Medicine, 2001. **33**(6): p. 682-7.

115. Bennett, P.N. and M.J. Brown, *Clinical pharmacology*. 10 ed. 2008, Edinburgh: Churchill Livingstone Elsevier.
116. Ricciotti, E. and G.A. FitzGerald, *Prostaglandins and inflammation.* Arteriosclerosis, Thrombosis, and Vascular Biology, 2011. **31**(5): p. 986-1000.
117. Klimp, A.H., H. Hollema, C. Kempinga, A.G.J. van der Zee, E.G.E. de Vries, and T. Daemen, *Expression of cyclooxygenase-2 and inducible nitric oxide synthase in human ovarian tumors and tumor-associated macrophages.* Cancer Research, 2001. **61**(19): p. 7305-7309.
118. Smith, G., R. Roberts, C. Hall, and G. Nuki, *Reversible ovulatory failure associated with the development of luteinized unruptured follicles in women with inflammatory arthritis taking non-steroidal anti-inflammatory drugs.* Rheumatology, 1996. **35**(5): p. 458-462.
119. Baldi, A., M. Campioni, and P.G. Signorile, *Endometriosis: pathogenesis, diagnosis, therapy and association with cancer (Review).* Oncology Reports, 2008. **19**(4): p. 843-846.
120. Van Gorp, T., F. Amant, P. Neven, I. Vergote, and P. Moerman, *Endometriosis and the development of malignant tumours of the pelvis. A review of literature.* Best Practice and Research: Clinical Obstetrics and Gynaecology, 2004. **18**(2): p. 349-371.
121. Worley, M.J., W.R. Welch, R.S. Berkowitz, and S.-W. Ng, *Endometriosis-associated ovarian cancer: a review of pathogenesis.* International Journal of Molecular Sciences, 2013. **14**(3): p. 5367-5379.
122. Kobayashi, H., *Ovarian cancer in endometriosis: epidemiology, natural history, and clinical diagnosis.* International Journal of Clinical Oncology, 2009. **14**(5): p. 378.
123. Ness, R.B. and F. Modugno, *Endometriosis as a model for inflammation-hormone interactions in ovarian and breast cancers.* European Journal of Cancer, 2006. **42**(6): p. 691-703.
124. Vlahos, N.F., T. Kalampokas, and S. Fotiou, *Endometriosis and ovarian cancer: a review.* Gynecological Endocrinology, 2010. **26**(3): p. 213-219.
125. Brinton, L.A., G. Gridley, I. Persson, J. Baron, and A. Bergqvist, *Cancer risk after a hospital discharge diagnosis of endometriosis.* American Journal of Obstetrics and Gynecology, 1997. **176**(3): p. 572-579.
126. Ness, R.B., *Endometriosis and ovarian cancer: thoughts on shared pathophysiology.* American Journal of Obstetrics and Gynecology, 2003. **189**(1): p. 280-294.

127. Braem, M.G.M., N.C. Onland-Moret, Pa, R.A. Goldbohm, P.H.M. Peeters, R. Kruitwagen, et al., *Reproductive and hormonal factors in association with ovarian cancer in the Netherlands cohort study.* American Journal of Epidemiology, 2010. **172**(10): p. 1181-1189.
128. Cibula, D., M. Widschwendter, M. Zikan, and L. Dusek, *Underlying mechanisms of ovarian cancer risk reduction after tubal ligation.* Acta Obstetricia et Gynecologica Scandinavica, 2011. **90**(6): p. 559-563.
129. Piek, J.M.J., P.J. van Diest, R.P. Zweemer, P. Kenemans, and R.H.M. Verheijen, *Tubal ligation and risk of ovarian cancer.* The Lancet, 2001. **358**(9284): p. 844-844.
130. Le, D.C., T. Kubo, Y. Fujino, D.C. Sokal, T.H. Vach, T.M. Pham, et al., *Reproductive factors in relation to ovarian cancer: A case-control study in Northern Vietnam.* Contraception, 2012. **86**(5): p. 494-499.
131. Rozenberg, S., J. Vandromme, and C. Antoine, *Postmenopausal hormone therapy: risks and benefits.* Nature Reviews Endocrinology, 2013. **9**(4): p. 216-227.
132. Writing Group for the Women's Health Initiative Investigators, *Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial.* JAMA, 2002. **288**(3): p. 321-333.
133. Zhou, B., Q. Sun, R. Cong, H. Gu, N. Tang, L. Yang, et al., *Hormone replacement therapy and ovarian cancer risk: A meta-analysis*. Gynecologic Oncology, 2008. **108**: p. 641-651.
134. Wernli, K.J., P.A. Newcomb, J.M. Hampton, A. Trentham-Dietz, and K.M. Egan, *Hormone therapy and ovarian cancer: incidence and survival.* Cancer Causes and Control, 2008. **19**(6): p. 605-613.
135. Pearce, C.L., K. Chung, M.C. Pike, and A.H. Wu, *Increased ovarian cancer risk associated with menopausal estrogen therapy is reduced by adding a progestin.* Cancer, 2009. **115**(3): p. 531-539.
136. Tsilidis, K.K., N.E. Allen, T.J. Key, L. Dossus, R. Kaaks, K. Bakken, et al., *Menopausal hormone therapy and risk of ovarian cancer in the European prospective investigation into cancer and nutrition.* Cancer Causes and Control, 2011. **22**(8): p. 1075-1084.
137. Hildebrand, J.S., S.M. Gapstur, H.S. Feigelson, L.R. Teras, M.J. Thun, and A.V. Patel, *Postmenopausal hormone use and incident ovarian cancer: associations differ by regimen.* International Journal of Cancer, 2010. **127**(12): p. 2928-2935.

138. Collaborative Group on Epidemiological Studies of Ovarian Cancer, *Menopausal hormone use and ovarian cancer risk: individual participant meta-analysis of 52 epidemiological studies.* The Lancet, 2015. **385**(9980): p. 1835-1842.
139. Morch, L.S., E. Lokkegaard, A.H. Andreasen, S.K. Kjaer, and O. Lidegaard, *Hormone therapy and different ovarian cancers: A national cohort study.* American Journal of Epidemiology, 2012. **175**(12): p. 1234-1242.
140. Morch, L.S., E. Lokkegaard, A.H. Andreasen, S.K. Kjaer, and O. Lidegaard, *Hormone therapy and ovarian borderline tumors: a national cohort study.* Cancer Causes and Control, 2012. **23**(1): p. 113-120.
141. Trabert, B., N. Wentzensen, H.P. Yang, M.E. Sherman, A. Hollenbeck, K.N. Danforth, et al., *Ovarian cancer and menopausal hormone therapy in the NIH-AARP diet and health study.* British Journal of Cancer, 2012. **107**(7): p. 1181-1187.
142. Beral, V., D. Bull, J. Green, and G. Reeves, *Ovarian cancer and hormone replacement therapy in the Million Women Study.* The Lancet, 2007. **369**(9574): p. 1703-10.
143. Mascarenhas, C., M. Lambe, R. Bellocco, K. Bergfeldt, T. Riman, I. Persson, et al., *Use of hormone replacement therapy before and after ovarian cancer diagnosis, and ovarian cancer survival.* International Journal of Cancer, 2006. **119**(12): p. 2907-2915.
144. Hill, A.B., *The environment and disease: association or causation?* Proceedings of the Royal Society of Medicine, 1965. **58**(5): p. 295.
145. Genkinger, J.M., D.J. Hunter, D. Spiegelman, K.E. Anderson, J.E. Buring, J.L. Freudenheim, et al., *Alcohol intake and ovarian cancer risk: a pooled analysis of 10 cohort studies.* British Journal of Cancer, 2006. **94**(5): p. 757-62.
146. Bagnardi, V., M. Blangiardo, C. La Vecchia, and G. Corrao, *A meta-analysis of alcohol drinking and cancer risk.* British Journal of Cancer, 2001. **85**(11): p. 1700.
147. Chang, E.T., A.J. Canchola, V.S. Lee, C.A. Clarke, D.M. Purdie, P. Reynolds, et al., *Wine and other alcohol consumption and risk of ovarian cancer in the California Teachers Study cohort.* Cancer Causes and Control, 2007. **18**(1): p. 91-103.
148. Schouten, L.J., M.P.A. Zeegers, R.A. Goldbohm, and P.A. van den Brandt, *Alcohol and ovarian cancer risk: results from the Netherlands Cohort Study.* Cancer Causes & Control, 2004. **15**(2): p. 201-9.

149. Modugno, F., R.B. Ness, and G.O. Allen, *Alcohol consumption and the risk of mucinous and nonmucinous epithelial ovarian cancer.* Obstetrics & Gynecology, 2003. **102**(6): p. 1336-43.

150. Green, A., D. Purdie, C. Bain, V. Siskind, and P.M. Webb, *Cigarette smoking and risk of epithelial ovarian cancer (Australia).* Cancer Causes & Control, 2001. **12**(8): p. 713-9.

151. Collaborative Group on Epidemiological Studies of Ovarian Cancer, V. Beral, K. Gaitskell, C. Hermon, K. Moser, G. Reeves, et al., *Ovarian cancer and smoking: individual participant meta-analysis including 28,114 women with ovarian cancer from 51 epidemiological studies.* Lancet Oncology, 2012. **13**(9): p. 946-56.

152. Purdie, D.M., P.M. Webb, V. Siskind, C.J. Bain, and A.C. Green, *The different etiologies of mucinous and nonmucinous epithelial ovarian cancers.* Gynecologic Oncology, 2003. **88**(1): p. S145-S148.

153. Marchbanks, P.A., H. Wilson, E. Bastos, D.W. Cramer, J.M. Schildkraut, and H.B. Peterson, *Cigarette smoking and epithelial ovarian cancer by histologic type.* Obstetrics & Gynecology, 2000. **95**(2): p. 255-60.

154. Kurian, A.W., R.R. Balise, V. McGuire, and A.S. Whittemore, *Histologic types of epithelial ovarian cancer: have they different risk factors?* Gynecologic Oncology, 2005. **96**(2): p. 520-30.

155. Willett, W., M.J. Stampfer, C. Bain, R. Lipnick, F.E. Speizer, B. Rosner, et al., *Cigarette smoking, relative weight, and menopause.* American Journal of Epidemiology, 1983. **117**(6): p. 651-658.

156. Lin, W.T., M. Beattie, L.-M. Chen, K. Oktay, S.L. Crawford, E.B. Gold, et al., *Comparison of age at natural menopause in BRCA1/2 mutation carriers with a non-clinic-based sample of women in northern California.* Cancer, 2013. **119**(9): p. 1652-9.

157. Engeland, A., S. Tretli, and T. Bjorge, *Height, body mass index, and ovarian cancer: a follow-up of 1.1 million Norwegian women.* Journal of the National Cancer Institute, 2003. **95**(16): p. 1244.

158. Schouten, L., J, R.A. Goldbohm, and P. van den Brandt, A, *Height, weight, weight change, and ovarian cancer risk in the Netherlands Cohort Study on Diet and Cancer.* American Journal of Epidemiology, 2003. **157**(5): p. 424.

159. Schouten, L.J., C. Rivera, D.J. Hunter, D. Spiegelman, H.-O. Adami, A. Arslan, et al., *Height, body mass index, and ovarian cancer: a pooled analysis of 12 cohort studies.* Cancer Epidemiology Biomarkers & Prevention, 2008. **17**(4): p. 902-912.

160. Beehler, G.P., M. Sekhon, J.A. Baker, and B.E. Teter, *Risk of ovarian cancer associated with BMI varies by menopausal status.* The Journal of Nutrition, 2006. **136**(11): p. 2881.

161. Rodriguez, C., E.E. Calle, D. Fakhrabadi-Shokoohi, E.J. Jacobs, and M.J. Thun, *Body mass index, height, and the risk of ovarian cancer mortality in a prospective cohort of postmenopausal women.* Cancer Epidemiology Biomarkers & Prevention, 2002. **11**(9): p. 822-828.

162. Collaborative Group on Epidemiological Studies of Ovarian Cancer, *Ovarian cancer and body size: individual participant meta-analysis including 25,157 women with ovarian cancer from 47 epidemiological studies.* PLoS Medicine / Public Library of Science, 2012. **9**(4): p. e1001200.

163. Leitzmann, M.F., C. Koebnick, K.N. Danforth, L.A. Brinton, S.C. Moore, A.R. Hollenbeck, et al., *Body mass index and risk of ovarian cancer.* Cancer, 2009. **115**(4): p. 812-822.

164. Lubin, F., A. Chetrit, L.S. Freedman, E. Alfandary, Y. Fishler, H. Nitzan, et al., *Body mass index at age 18 years and during adult life and ovarian cancer risk.* American Journal of Epidemiology, 2003. **157**(2): p. 113-20.

165. Rossing, M.A., M.-T.C. Tang, E.W. Flagg, L.K. Weiss, K.G. Wicklund, and N.S. Weiss, *Body size and risk of epithelial ovarian cancer (United States).* Cancer Causes & Control, 2006. **17**(5): p. 713-20.

166. Purdie, D.M., C.J. Bain, P.M. Webb, D.C. Whiteman, S. Pirozzo, and A.C. Green, *Body size and ovarian cancer: case–control study and systematic review (Australia).* Cancer Causes and Control, 2001. **12**(9): p. 855-863.

167. Greer, J.B., F. Modugno, R.B. Ness, and G.O. Allen, *Anthropometry and the risk of epithelial ovarian cancer.* Cancer, 2006. **106**(10): p. 2247-57.

168. Miles, L., *Physical activity and the prevention of cancer: a review of recent findings.* Nutrition Bulletin, 2007. **32**(3): p. 250-282.

169. Leitzmann, M.F., C. Koebnick, S.C. Moore, K.N. Danforth, L.A. Brinton, A.R. Hollenbeck, et al., *Prospective study of physical activity and the risk of ovarian cancer.* Cancer Causes & Control, 2009. **20**(5): p. 765-73.

170. Bertone, E.R., W.C. Willett, B.A. Rosner, D.J. Hunter, C.S. Fuchs, F.E. Speizer, et al., *Prospective study of recreational physical activity and ovarian cancer.* Journal of the National Cancer Institute, 2001. **93**(12): p. 942-8.
171. Cottreau, C.M., R.B. Ness, and A.M. Kriska, *Physical activity and reduced risk of ovarian cancer.* Obstetrics & Gynecology, 2000. **96**(4): p. 609-14.
172. Rice, L.W., *Hormone prevention strategies for breast, endometrial and ovarian cancers.* Gynecologic Oncology, 2010. **118**(2): p. 202-7.
173. Finch, A., G. Evans, and S.A. Narod, *BRCA carriers, prophylactic salpingo-oophorectomy and menopause: clinical management considerations and recommendations.* Women's Health, 2012. **8**(5): p. 543-555.
174. Gulati, A.P. and S.M. Domchek, *The clinical management of BRCA1 and BRCA2 mutation carriers.* Current Oncology Reports, 2008. **10**(1): p. 47-53.
175. Hanson, H. and S. Hodgson, *Cancer genetics and reproduction.* Best Practice & Research: Clinical Obstetrics & Gynaecology, 2010. **24**(1): p. 3-18.
176. Lancaster, J.M., C.B. Powell, N.D. Kauff, I. Cass, L.-M. Chen, K.H. Lu, et al., *Society of Gynecologic Oncologists Education Committee statement on risk assessment for inherited gynecologic cancer predispositions.* Gynecologic Oncology, 2007. **107**(2): p. 159-162.
177. Pepe, S., M. Pensabene, and C. Condello, *Modifiers of risk in BRCA1/2 mutation carriers.* Current Bioactive Compounds, 2012. **8**(1): p. 23-29.
178. Schmeler, K.M. and K.H. Lu, *Gynecologic cancers associated with Lynch syndrome/HNPCC.* Clinical and Translational Oncology, 2008. **10**(6): p. 313-317.
179. Levy, D., J. Garber, and A. Shields, *Guidelines for genetic risk assessment of hereditary breast and ovarian cancer: early disagreements and low utilization.* Journal of General Internal Medicine, 2009. **24**(7): p. 822.
180. Bernholtz, S., Y. Laitman, B. Kaufman, S. Paluch Shimon, and E. Friedman, *Cancer risk in Jewish BRCA1 and BRCA2 mutation carriers: effects of oral contraceptive use and parental origin of mutation.* Breast Cancer Research and Treatment, 2011. **129**(2): p. 557-63.
181. McGuire, V., A. Felberg, M. Mills, K.L. Ostrow, R. DiCioccio, E.M. John, et al., *Relation of contraceptive and reproductive history to ovarian cancer risk in carriers and noncarriers of BRCA1 gene mutations.* American Journal of Epidemiology, 2004. **160**(7): p. 613-618.

182. Antoniou, A.C., M. Rookus, N. Andrieu, R. Brohet, J. Chang-Claude, S. Peock, et al., *Reproductive and hormonal factors, and ovarian cancer risk for BRCA1 and BRCA2 mutation carriers: results from the International BRCA1/2 Carrier Cohort Study.* Cancer Epidemiology, Biomarkers and Prevention, 2009. **18**(2): p. 601-10.

183. Hong, H., H.Y. Yen, A. Brockmeyer, Y. Liu, R. Chodankar, M.C. Pike, et al., *Changes in the mouse estrus cycle in response to Brca1 inactivation suggest a potential link between risk factors for familial and sporadic ovarian cancer.* Cancer Research, 2010. **70**(1): p. 221-228.

184. Tung, K.-H., M.T. Goodman, A.H. Wu, K. McDuffie, L.R. Wilkens, A.M.Y. Nomura, et al., *Aggregation of ovarian cancer with breast, ovarian, colorectal, and prostate cancer in first-degree relatives.* American Journal of Epidemiology, 2004. **159**(8): p. 750-8.

185. Ellberg, C., H. Jernström, P. Broberg, Å. Borg, and H. Olsson, *Impact of a paternal origin of germline BRCA1/2 mutations on the age at breast and ovarian cancer diagnosis in a Southern Swedish cohort.* Genes, Chromosomes and Cancer, 2015. **54**(1): p. 39-50.

186. Yang, H.P., B. Trabert, M.A. Murphy, M.E. Sherman, J.N. Sampson, L.A. Brinton, et al., *Ovarian cancer risk factors by histologic subtypes in the NIH-AARP Diet and Health Study.* International Journal of Cancer, 2012. **131**(4): p. 938-48.

187. Boyce, E.A., I. Costaggini, A. Vitonis, C. Feltmate, M. Muto, R. Berkowitz, et al., *The epidemiology of ovarian granulosa cell tumors: a case-control study.* Gynecologic Oncology, 2009. **115**(2): p. 221-5.

188. Parazzini, F., F. Chiaffarino, E. Negri, M. Surace, G. Benzi, S. Franceschi, et al., *Risk factors for different histological types of ovarian cancer.* International Journal of Gynecological Cancer, 2004. **14**(3): p. 431-6.

189. Purdie, D.M., V. Siskind, C.J. Bain, P.M. Webb, and A.C. Green, *Reproduction-related risk factors for mucinous and nonmucinous epithelial ovarian cancer.* American Journal of Epidemiology, 2001. **153**(9): p. 860-4.

190. Risch, H.A., L.D. Marrett, M. Jain, and G.R. Howe, *Differences in risk factors for epithelial ovarian cancer by histologic type. Results of a case-control study.* American Journal of Epidemiology, 1996. **144**(4): p. 363-72.

191. Gates, M., B. Rosner, J. Hecht, and S. Tworoger, *Risk factors for epithelial ovarian cancer by histologic subtype.* American Journal of Epidemiology, 2010. **171**(1): p. 45.

192. Tung, K.-H., M.T. Goodman, A.H. Wu, K. McDuffie, L.R. Wilkens, L.N. Kolonel, et al., *Reproductive factors and epithelial ovarian cancer risk by histologic type: a multiethnic case-control study.* American Journal of Epidemiology, 2003. **158**(7): p. 629-638.

193. Nagle, C.M., C.M. Olsen, P.M. Webb, S.J. Jordan, D.C. Whiteman, and A.C. Green, *Endometrioid and clear cell ovarian cancers: a comparative analysis of risk factors.* European Journal of Cancer, 2008. **44**(16): p. 2477-2484.

194. Jordan, S.J., A.C. Green, D.C. Whiteman, S.P. Moore, C.J. Bain, D.M. Gertig, et al., *Serous ovarian, fallopian tube and primary peritoneal cancers: a comparative epidemiological analysis.* International Journal of Cancer, 2008. **122**(7): p. 1598-603.

195. Bray, F., A.H. Loos, S. Tognazzo, and C. La Vecchia, *Ovarian cancer in Europe: cross-sectional trends in incidence and mortality in 28 countries, 1953-2000.* International Journal of Cancer, 2005. **113**(6): p. 977-90.

196. Laurvick, C.L., J.B. Semmens, C.D.A.J. Holman, and Y.C. Leung, *Ovarian cancer in Western Australia (1982-98): incidence, mortality and survival.* Australian and New Zealand Journal of Public Health, 2003. **27**(6): p. 588-95.

197. Firestone, R.T., K.C. Wong, L. Ellison-Loschmann, and M. Jeffreys, *Characteristics of ovarian cancer in women residing in Aotearoa, New Zealand: 1993-2004.* Journal of Epidemiology and Community Health, 2009. **63**: p. 814-9.

198. dos Santos Silva, I. and A.J. Swerdlow, *Recent trends in incidence of and mortality from breast, ovarian and endometrial cancers in England and Wales and their relation to changing fertility and oral contraceptive use.* British Journal of Cancer, 1995. **72**(2): p. 485-92.

199. Collaborative Group on Epidemiological Studies of Ovarian Cancer, V. Beral, R. Doll, C. Hermon, R. Peto, and G. Reeves, *Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls.* The Lancet, 2008. **371**(9609): p. 303-14.

200. Skirnisdottir, I., H. Garmo, E. Wilander, and L. Holmberg, *Borderline ovarian tumors in Sweden 1960-2005: trends in incidence and age at diagnosis compared to ovarian cancer.* International Journal of Cancer, 2008. **123**(8): p. 1897-901.

201. Jeffreys, M., V. Stevanovic, M. Tobias, C. Lewis, L. Ellison-Loschmann, N. Pearce, et al., *Ethnic inequalities in cancer survival in New Zealand: linkage study.* American Journal of Public Health, 2005. **95**(5): p. 834-837.

202. Siegel, R., D. Naishadham, and A. Jemal, *Cancer statistics, 2012.* CA: A Cancer Journal for Clinicians, 2012. **62**(1): p. 10-29.
203. Mai, P.L., N. Wentzensen, and M.H. Greene, *Challenges related to developing serum-based biomarkers for early ovarian cancer detection.* Cancer Prevention Research, 2011. **4**(3): p. 303-306.
204. van Nagell, J.R., R.W. Miller, C.P. DeSimone, F.R. Ueland, I. Podzielinski, S.T. Goodrich, et al., *Long-term survival of women with epithelial ovarian cancer detected by ultrasonographic screening.* Obstetrics and Gynecology, 2011. **118**(6): p. 1212-1221.
205. Goff, B.A., L.S. Mandel, C.W. Drescher, N. Urban, S. Gough, K.M. Schurman, et al., *Development of an ovarian cancer symptom index.* Cancer, 2007. **109**(2): p. 221-227.
206. Markman, M., *Development of an ovarian cancer symptom index: possibilities for earlier detection.* Cancer, 2007. **110**(1): p. 226-227.
207. Dorigo, O. and J.S. Berek, *Personalizing CA125 levels for ovarian cancer screening.* Cancer Prevention Research, 2011. **4**(9): p. 1356-9.
208. Skates, S.J., P. Mai, N.K. Horick, M. Piedmonte, C.W. Drescher, C. Isaacs, et al., *Large prospective study of ovarian cancer screening in high-risk women: CA125 cut-point defined by menopausal status.* Cancer Prevention Research, 2011. **4**(9): p. 1401-8.
209. Jacobs, I., D. Oram, J. Fairbanks, J. Turner, C. Frost, and J.G. Grudzinskas, *A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer.* BJOG: An International Journal of Obstetrics & Gynaecology, 1990. **97**(10): p. 922-929.
210. Obeidat, B.R., Z.O. Amarin, J.A. Latimer, and R.A. Crawford, *Risk of malignancy index in the preoperative evaluation of pelvic masses.* International Journal of Gynecology & Obstetrics, 2004. **85**(3): p. 255-258.
211. Jacobs, I.J., U. Menon, A. Ryan, A. Gentry-Maharaj, M. Burnell, J.K. Kalsi, et al., *Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial.* The Lancet, 2015.
212. Lu, K.H. and M. Daniels, *Endometrial and ovarian cancer in women with Lynch syndrome: update in screening and prevention.* Familial Cancer, 2013. **12**(2): p. 273-277.
213. Finch, A., K.A. Metcalfe, J.K. Chiang, L. Elit, J. McLaughlin, C. Springate, et al., *The impact of prophylactic salpingo-oophorectomy on menopausal symptoms and sexual*

function in women who carry a BRCA mutation. Gynecologic Oncology, 2011. **121**(1): p. 163-168.

214. Vitonis, A.F., L. Titus-Ernstoff, and D.W. Cramer, *Assessing ovarian cancer risk when considering elective oophorectomy at the time of hysterectomy.* Obstetrics and Gynecology, 2011. **117**(5): p. 1042-50.
215. Kobel, M., S.E. Kalloger, J.L. Santos, D.G. Huntsman, C.B. Gilks, and K.D. Swenerton, *Tumor type and substage predict survival in stage I and II ovarian carcinoma: Insights and implications.* Gynecologic Oncology, 2010. **116**(1): p. 50-56.
216. Nagle, C.M., C.J. Bain, A.C. Green, and P.M. Webb, *The influence of reproductive and hormonal factors on ovarian cancer survival.* International Journal of Gynecological Cancer, 2008. **18**(3): p. 407-13.
217. Yang, L., A. Klint, M. Lambe, R. Bellocco, T. Riman, K. Bergfeldt, et al., *Predictors of ovarian cancer survival: a population-based prospective study in Sweden.* International Journal of Cancer, 2008. **123**(3): p. 672-9.
218. Zhang, M. and C.D.A.J. Holman, *Tubal ligation and survival of ovarian cancer patients.* Journal of Obstetrics and Gynaecology Research, 2012. **38**(1): p. 40-7.
219. Li, W., X. Wu, C.Q. Fang, J.F. Yao, Y. Guo, and S.L. Zhang, *Prognostic factors in adult granulosa cell tumor of the ovary.* Saudi Medical Journal, 2009. **30**(2): p. 247-252.
220. Previs, R., J. Kilgore, R. Craven, G. Broadwater, S. Bean, S. Wobker, et al., *Obesity is associated with worse overall survival in women with low grade papillary serous epithelial ovarian cancer.* International Journal of Gynecological Cancer: Official Journal of the International Gynecological Cancer Society, 2014. **24**(4): p. 670.
221. Nagle, C.M., C.J. Bain, and P.M. Webb, *Cigarette smoking and survival after ovarian cancer diagnosis.* Cancer Epidemiology Biomarkers & Prevention, 2006. **15**(12): p. 2557-2560.
222. Dutta, D.C., *Textbook of gynaecology*. 4th ed. 2003, Calcutta: New Central Book Agency.
223. Van der Westhuizen, E. and E. Van der Merwe, *The safety and efficacy of low-dose oral contraceptives.* South African Family Practice, 2011. **53**(5): p. 403-411.
224. Wiegratz, I. and C.J. Thaler, *Hormonal contraception: what kind, when, and for whom?* Deutsches Arzteblatt, 2011. **108**(28-29): p. 495-506.

225. Ministry of Public Health and Sanitation, *National Family Planning guidelines for service providers*. 2010, Division of Reproductive Health, Ministry of Public Health and Sanitation, Kenya: Nairobi.
226. Eshre Capri Workshop Group, *Family planning 2011: better use of existing methods, new strategies and more informed choices for female contraception.* Human Reproduction Update, 2012. **18**(6): p. 670-81.
227. Mansour, D., P. Inki, and K. Gemzell-Danielsson, *Efficacy of contraceptive methods: a review of the literature.* The European Journal of Contraception and Reproductive Health Care, 2010(15): p. S19-S31.
228. Trussell, J., *Contraceptive failure in the United States.* Contraception, 2004. **70**(2): p. 89-96.
229. Zureick-Brown, S., H. Newby, D. Chou, N. Mizoguchi, L. Say, E. Suzuki, et al., *Understanding global trends in maternal mortality.* International Perspective on Sexual Reproduction and Health, 2013. **39**(1): p. 32-41.
230. Cunat, S., P. Hoffmann, and P. Pujol, *Estrogens and epithelial ovarian cancer.* Gynecologic Oncology, 2004. **94**(1): p. 25-32.
231. Cibula, D., A. Gompel, A.O. Mueck, C. La Vecchia, P.C. Hannaford, S.O. Skouby, et al., *Hormonal contraception and risk of cancer.* Human Reproduction Update, 2010. **16**(6): p. 631-650.
232. Vineis, P., A. Schatzkin, and J.D. Potter, *Models of carcinogenesis: an overview.* Carcinogenesis, 2010. **31**(10): p. 1703-1709.
233. Henderson, B.E., R.K. Ross, M.C. Pike, and J.T. Casagrande, *Endogenous hormones as a major factor in human cancer.* Cancer Research, 1982. **42**(8): p. 3232-3239.
234. Vakkila, J. and M.T. Lotze, *Inflammation and necrosis promote tumour growth.* Nature Reviews Immunology, 2004. **4**(8): p. 641-648.
235. Saad, A.F., W. Hu, and A.K. Sood, *Microenvironment and pathogenesis of epithelial ovarian cancer.* Hormones and Cancer, 2010. **1**(6): p. 277-290.
236. Nasca, P.C. and H. Pastides, eds. *Fundamentals of cancer epidemiology*. Second ed.'Nasca, P.C. and H. Pastidess'. Nasca, P.C. and H. Pastides. 2008, Jones and Bartlett Publishers: Sudbury, MA.
237. Key, T.J., N.E. Allen, P.K. Verkasalo, and E. Banks, *Energy balance and cancer: The role of sex hormones.* Proceedings of the Nutrition Society, 2001. **60**(1): p. 81-89.
238. Syed, V., G. Ulinski, S.C. Mok, G.K. Yiu, and S.M. Ho, *Expression of gonadotropin receptor and growth responses to key reproductive hormones in normal and*

malignant human ovarian surface epithelial cells. Cancer Research, 2001. **61**(18): p. 6768-6776.

239. Lukanova, A. and R. Kaaks, *Endogenous hormones and ovarian cancer: epidemiology and current hypotheses.* Cancer Epidemiology Biomarkers and Prevention, 2005. **14**(1): p. 98-107.
240. Grimbizis, G.F. and B.C. Tarlatzis, *The use of hormonal contraception and its protective role against endometrial and ovarian cancer.* Best Practice & Research: Clinical Obstetrics & Gynaecology, 2010. **24**(1): p. 29-38.
241. Dorjgochoo, T., X.O. Shu, H.L. Li, H.Z. Qian, G. Yang, H. Cai, et al., *Use of oral contraceptives, intrauterine devices and tubal sterilization and cancer risk in a large prospective study, from 1996 to 2006.* International Journal of Cancer, 2009. **124**(10): p. 2442-2449.
242. Clinton, G.M. and W. Hua, *Estrogen action in human ovarian cancer.* Critical Reviews in Oncology/Hematology, 1997. **25**(1): p. 1-9.
243. Rodriguez, G.C., N.P. Nagarsheth, K.L. Lee, R.C. Bentley, D.K. Walmer, M. Cline, et al., *Progestin-induced apoptosis in the Macaque ovarian epithelium: differential regulation of transforming growth factor-beta.* Journal of the National Cancer Institute, 2002. **94**(1): p. 50-60.
244. Murdoch, W.J., *Carcinogenic potential of ovulatory genotoxicity.* Biology of Reproduction, 2005. **73**(4): p. 586-590.
245. Choi, J.H., A.S.T. Wong, H.F. Huang, and P.C.K. Leung, *Gonadotropins and ovarian cancer.* Endocrine Reviews, 2007. **28**(4): p. 440-461.
246. Gadducci, A., S. Cosio, A. Gargini, and A.R. Genazzani, *Sex-steroid hormones, gonadotropin and ovarian carcinogenesis: A review of epidemiological and experimental data.* Gynecological Endocrinology, 2004. **19**(4): p. 216-228.
247. Heller, D.S., P. Murphy, and C. Westhoff, *Are germinal inclusion cysts markers of ovulation?* Gynecologic Oncology, 2005. **96**(2): p. 496-9.
248. Rodriguez, G.C., D.K. Walmer, M. Cline, H. Krigman, B.A. Lessey, R.S. Whitaker, et al., *Effect of progestin on the ovarian epithelium of macaques: cancer prevention through apoptosis?* Journal of the Society for Gynecologic Investigation, 1998. **5**(5): p. 271-6.
249. Murdoch, W. and A. McDonnel, *Roles of the ovarian surface epithelium in ovulation and carcinogenesis.* Reproduction, 2002. **123**(6): p. 743-750.

250. King, S.M., T.S. Hilliard, L.Y. Wu, R.C. Jaffe, A.T. Fazleabas, and J.E. Burdette, *The impact of ovulation on fallopian tube epithelial cells: evaluating three hypotheses connecting ovulation and serous ovarian cancer*. Endocrine-Related Cancer, 2011. **18**(5): p. 627-42.

251. Saleemuddin, A., A.K. Folkins, L. Garrett, J. Garber, M.G. Muto, C.P. Crum, et al., *Risk factors for a serous cancer precursor ("p53 signature") in women with inherited BRCA mutations.* Gynecologic Oncology, 2008. **111**(2): p. 226-32.

252. Pearce, C.L., C. Templeman, M. Rossing, Anne, A. Lee, A.M. Near, and P. Webb, M, *Association between endometriosis and risk of histological subtypes of ovarian cancer: a pooled analysis of case-control studies.* Lancet Oncology, 2012. **13**(4): p. 385-394.

253. Potter, J.D., *Morphogens, morphostats, microarchitecture and malignancy.* Nature Reviews Cancer, 2007. **7**(6): p. 464-474.

254. Halperin, R., E. Hadas, R. Langer, I. Bukovsky, and D. Schneider, *Peritoneal fluid gonadotropins and ovarian hormones in patients with ovarian cancer.* International Journal of Gynecological Cancer, 1999. **9**(6): p. 502-507.

255. Ho, S.M., *Estrogen, progesterone and epithelial ovarian cancer.* Reproductive Biology and Endocrinology, 2003. **1**(73).

256. Bu, S.Z., D.L. Yin, X.H. Ren, L.Z. Jiang, Z.J. Wu, Q.R. Gao, et al., *Progesterone induces apoptosis and up-regulation of p53 expression in human ovarian carcinoma cell lines.* Cancer, 1997. **79**(10): p. 1944-1950.

257. Gogoi, R., M. Kudla, O. Gil, and D. Fishman, *The activity of medroxyprogesterone acetate, an androgenic ligand, in ovarian cancer cell invasion.* Reproductive Sciences, 2008. **15**(8): p. 846-52.

258. Thun, M.J., S.J. Henley, and T. Gansler. *Inflammation and cancer: an epidemiological perspective*. in *Novartis Foundation Symposium*. 2004. Chichester; New York; John Wiley.

259. Lawrence, T., *Inflammation and cancer: a failure of resolution?* Trends in Pharmacological Sciences, 2007. **28**(4): p. 162-165.

260. Mann, T. and C. Lutwak-Mann, *Male reproductive function and semen: themes and trends in physiology, biochemistry and investigative andrology*. 1981, Berlin: Springer-Verlag.

261. Arienti, G., C. Saccardi, E. Carlini, R. Verdacchi, and C.A. Palmerini, *Distribution of lipid and protein in human semen fractions.* Clinica Chimica Acta, 1999. **289**(1): p. 111-120.

262. Solomon, B. and M.W. Duncan, *Proteomics of seminal fluid*, in *Proteomics of Human Body Fluids*. 2007. New York, Springer. p. 467-493.

263. Robertson, S.A., *Seminal plasma and male factor signalling in the female reproductive tract.* Cell and Tissue Research, 2005. **322**(1): p. 43-52.

264. Gillott, C., *Male accessory gland secretions: modulators of female reproductive physiology and behavior.* Annual Review of Entomology, 2003. **48**(1): p. 163-184.

265. Naik, V., A. Thakur, A. Sheth, U. Joshi, S.S. Rao, D. Pardanani, et al., *The effect of vasectomy on pituitary-gonadal function in men.* Journal of Reproduction and Fertility, 1976. **48**(2): p. 441-442.

266. Jakobsen, H., H. Rui, Y. Thomassen, T. Hald, and K. Purvis, *Polyamines and other accessory sex gland secretions in human seminal plasma 8 years after vasectomy.* Journal of Reproduction and Fertility, 1989. **87**(1): p. 39-45.

267. Thakur, A., A. Sheth, S. Rao, and P. Thacker, *Effect of vasectomy on the prostatic function as indicated by seminal maltase activity.* Contraception, 1975. **11**(2): p. 155-159.

268. Naik, V., U. Joshi, and A. Sheth, *Long-term effects of vasectomy on prostatic function in men.* Journal of Reproduction and Fertility, 1980. **58**(2): p. 289-293.

269. Bendvold, E., K. Svanborg, M. Bygdeman, and S. Noren, *On the origin of prostaglandins in human seminal fluid.* International Journal of Andrology, 1985. **8**(1): p. 37-43.

270. Ying, W., M. Hedman, B. Torre, F. Jensen, P. Pedersen, and E. Diczfalusy, *Effect of vasectomy on the steroid profile of human seminal plasma.* International Journal of Andrology, 1983. **6**(2): p. 116-124.

271. Fernandez, C., R. Sharrard, M. Talbot, B. Reed, and N. Monks, *Evaluation of the significance of polyamines and their oxidases in the aetiology of human cervical carcinoma.* British Journal of Cancer, 1995. **72**(5): p. 1194.

272. Calle, E., C. Heath, H. MiracleMcMahill, R. Coates, J. Liff, S. Franceschi, et al., *Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies.* The Lancet, 1996. **347**(9017): p. 1713-27.

273. Vessey, M., D. Yeates, and S. Flynn, *Factors affecting mortality in a large cohort study with special reference to oral contraceptive use.* Contraception, 2010. **82**(3): p. 221-9.
274. Bosch, F.X. and S. de Sanjosé, *The epidemiology of human papillomavirus infection and cervical cancer.* Disease Markers, 2007. **23**(4): p. 213-227.
275. Van der Westhuizen, E. and E. Van der Merwe, *The safety and efficacy of low-dose oral contraceptives.* SA Pharmaceutical Journal, 2010. **77**(1): p. 22-28.
276. Potter, J.D. and A.J. McMichael, *Large bowel cancer in women in relation to reproductive and hormonal factors: a case-control study.* Journal of the National Cancer Institute, 1983. **71**(4): p. 703-709.
277. Kaunitz, A.M., *Depot medroxyprogesterone acetate contraception and the risk of breast and gynecologic cancer.* Journal of Reproductive Medicine for the Obstetrician and Gynecologist, 1996. **41**(5 SUPPL.): p. 419-427.
278. Paul, C., D.C. Skegg, and G.F. Spears, *Depot medroxyprogesterone (Depo-Provera) and risk of breast cancer.* BMJ (Clinical Research Ed.), 1989. **299**: p. 759-762.
279. Bakry, S., Z.O. Merhi, T.J. Scalise, M.S. Mahmoud, A. Fadiel, and F. Naftolin, *Depot-medroxyprogesterone acetate: an update.* Archives of Gynecology and Obstetrics, 2008. **278**(1): p. 1-12.
280. Hannaford, P.C., L. Iversen, T.V. Macfarlane, A.M. Elliott, V. Angus, and A.J. Lee, *Mortality among contraceptive pill users: cohort evidence from Royal College of General Practitioners' Oral Contraception Study.* BMJ (Clinical Research Ed.), 2010. **340**: p. c927.
281. dos Santos Silva, I., *Cancer epidemiology: principles and methods*. Second edition ed. 1999, Lyon: International Agency for Research on Cancer.
282. Rothman, K.J., *Epidemiology: an introduction*. Second ed. 2012, New York, NY: Oxford University Press.
283. Cox, B. and M.J. Sneyd, *School milk and risk of colorectal cancer: a national case-control study.* American Journal of Epidemiology, 2011. **173**(4): p. 394-403.
284. Cox, B., M.J. Sneyd, C. Paul, and D.C.G. Skegg, *Risk factors for prostate cancer: a national case-control study.* International Journal of Cancer, 2006. **119**(7): p. 1690-1694.
285. Statistics New Zealand. *2013 census usually resident population counts*. 2013 [Accessed on 20/05/2016] Available from:

http://www.stats.govt.nz/browse_for_stats/population/census_counts/2013CensusUsuallyResidentPopulationCounts_HOTP2013Census.aspx.

286. Statistics New Zealand. *2013 census: major ethnic groups in New Zealand*. 2015 29 January 2015 [Accessed on 20/05/2016] Available from: http://www.stats.govt.nz/Census/2013-census/profile-and-summary-reports/infographic-culture-identity.aspx.

287. Statistics New Zealand. *Language*. 2015 2 October 2014 [Accessed on 22/05/2016] Available from: http://www.stats.govt.nz/browse_for_stats/people_and_communities/Language.aspx.

288. Sneyd, M.J., B. Cox, C. Paul, and D.C.G. Skegg, *High prevalence of vasectomy in New Zealand.* Contraception, 2001. **64**(3): p. 155-159.

289. Ministry of Health, *Cancer: new registrations and deaths 2009*. 2012, Wellington, New Zealand: Ministry of Health.

290. Rosner, B., *Fundamentals of biostatistics*. 7 ed. 2011, Boston MA,: Cengage Learning.

291. Jewell, N.P., *Statistics for epidemiology*. 2004, Boca Raton: CRC Press.

292. Council for International Organizations of Medical Sciences (CIOMS) and World Health Organization (WHO), *International ethical guidelines for epidemiological studies*. 2008, CIOMS: Geneva.

293. New Zealand Post Ltd. *Address and Postcode Finder*. 2016 [Accessed on 3rd March] Available from: https://www.nzpost.co.nz.

294. Yusuf, F. and S. Siedlecky, *Contraceptive use in Australia: evidence from the 1995 National Health Survey*. Australian and New Zealand Journal of Obstetrics and Gynaecology, 1999. **39**(1): p. 58-62.

295. Dharmalingam, A., I. Pool, J. Sceats, and R. Mackay, *Patterns of family formation and change in New Zealand*. 2004: Centre for Social Research and Evaluation, Ministry of Social Development Wellington.

296. Statistics New Zealand. *Births and Deaths: Year ended December 2014*. 2015 [Accessed on 7th November] Available from: http://www.stats.govt.nz/browse_for_stats/population/births/BirthsAndDeaths_HOTPYeDec14/Commentary.aspx.

297. Fanslow, J., A. Whitehead, M. Silva, and E. Robinson, *Contraceptive use and associations with intimate partner violence among a population-based sample of New*

Zealand women. Australian and New Zealand Journal of Obstetrics and Gynaecology, 2008. **48**: p. 83-89.

298. Dharmalingam, A., I. Pool, and J. Sceats, *A demographic history of the New Zealand family from 1840: tables*. 2007, Auckland University.
299. Jones, J., W. Mosher, and K. Daniels, *Current contraceptive use in the United States, 2006-2010, and changes in patterns of use since 1995.* National health statistics reports, 2012(60): p. 1.
300. Skouby, S.O., *Contraceptive use and behavior in the 21st century: a comprehensive study across five European countries.* European J.of Contraception and Reproductive Healthcare, 2004. **9**(2): p. 57-68.
301. Paul, C., D.C.G. Skegg, and S. Williams, *Depot medroxyprogesterone acetate: Patterns of use and reasons for discontinuation.* Contraception, 1997. **56**(4): p. 209-214.
302. Piccinino, L.J. and W.D. Mosher, *Trends in contraceptive use in the United States: 1982-1995.* Family Planning Perspectives, 1998. **30**(1): p. 4-46.
303. Paul, S., M. Tobias, and C. Wright, *Setting outcome targets for the National Cervical Screening Programme.* A Report for the National Screening Unit. Wellington, New Zealand: Ministry of Health, 2005.
304. Statistics New Zealand. *2013 census quickstats about transport and communications*. 2015 03 February 2015 [Accessed on 25/05/2016] Available from: http://www.stats.govt.nz/Census/2013-census/profile-and-summary-reports/quickstats-transport-comms/access-telecomms.aspx.
305. Hayes, J., A. Richardson, and C. Frampton, *Population attributable risks for modifiable lifestyle factors and breast cancer in New Zealand women.* Internal Medicine Journal, 2013. **43**(11): p. 1198-1204.
306. Anuurad, E., K. Shiwaku, A. Nogi, K. Kitajima, B. Enkhmaa, K. Shimono, et al., *The new BMI criteria for asians by the regional office for the western pacific region of WHO are suitable for screening of overweight to prevent metabolic syndrome in elder Japanese workers.* Journal of Occupational Health, 2003. **45**(6): p. 335-343.
307. Sneyd, M.J. and B. Cox, *Commentary: Decreasing response rates require investigators to quantify and report the impact of selection bias in case–control studies.* International Journal of Epidemiology, 2011. **40**(5): p. 1355-1357.
308. Pandeya, N., G.M. Williams, A.C. Green, P.M. Webb, and D.C. Whiteman, *Do low control response rates always affect the findings? Assessments of smoking and obesity*

in two Australian case-control studies of cancer. Australian and New Zealand Journal of Public Health, 2009. **33**(4): p. 312-319.

309. Ibiebele, T., C. Nagle, C. Bain, and P.M. Webb, *Intake of omega-3 and omega-6 fatty acids and risk of ovarian cancer.* Cancer Causes and Control, 2012. **23**(11): p. 1775-1783.

310. Ministry of Education. *The Adult Literacy and Life Skills (ALL) Survey: overview and international comparisons*. [Accessed on 24/05/2013] Available from: http://www.educationcounts.govt.nz/publications/literacy/19491.

311. Webb, P. and C. Bain, *Essential epidemiology: an introduction for students and health professionals*. 2 ed. 2011, Cambridge: Cambridge University Press.

APPENDICES

APPENDIX 1: SEARCH STRATEGY

Embase

1. ovary cancer/ or ovary tumor/ or dysgerminoma/ or granulosa cell tumor/ or "hereditary breast and ovarian cancer syndrome"/ or ovary adenocarcinoma/ or ovary carcinoma/
2. ((ovarian or ovary or ovaries) adj3 (cancer* or tumour* or tumor* or neoplasm* or malignan*)).tw.
3. 1 or 2
4. vasectomy/
5. vasectomy.tw.
6. exp intrauterine contraceptive device/
7. (intrauterine adj3 contracept*).tw.
8. injectable contraceptive agent/ or estradiol cypionate plus medroxyprogesterone acetate/ or estradiol enanthate/ or estradiol valerate plus norethisterone enantate/ or medroxyprogesterone acetate/ or norethisterone enantate/
9. medroxyprogesterone/
10. (contraception adj3 implant*).tw.
11. or/4-10
12. risk.tw.
13. cancer risk/
14. exp risk/
15. 12 or 13 or 14
16. 3 and 11 and 15
17. letter.pt.
18. editorial.pt.
19. 16 not (17 or 18)
20. limit 19 to english language

Medline

1. ovarian neoplasms/ or granulosa cell tumor/ or "hereditary breast and ovarian cancer syndrome"/ or luteoma/ or sertoli-leydig cell tumor/ or thecoma/
2. ((ovarian or ovary or ovaries) adj3 (cancer* or tumour* or tumor* or neoplasm* or carcinoma* or malignan*)).tw.
3. 1 or 2
4. exp Contraceptives, Oral/
5. exp contraception/
6. Vasectomy/
7. vasectomy.tw.
8. oral contracept*.tw.
9. 4 or 5 or 6 or 7 or 8
10. 3 and 9
11. letter.pt.
12. editorial.pt.
13. 11 or 12
14. 10 not 13
15. limit 14 to english language

CINAHL

S1. (MH "Ovarian Neoplasms+")

S2. ((ovarian or ovary or ovaries)) AND ((cancer* or tumour* or tumor* or neoplasm* or carcinoma* or malignan*))

S3. S1 OR S2

S4. (MH "Contraceptives, Oral+")

S5. (MH "Contraception+")

S6. (MH "Intrauterine Devices")

S7. (MH "Vasectomy")

S8. vasectomy

S9. (MH "Medroxyprogesterone+")

S10. S4 OR S5 OR S6 OR S7 OR S8 OR S9

S11. S3 AND S10

S12. S3 AND S10

S13. (MH "Relative Risk")

S14. (MH "Risk Assessment")

S15. risk

S16. S13 OR S14 OR S15

S17. S12 AND S16

APPENDIX 2: LETTER TO DOCTORS

School of Health Sciences

Tel: +64 3 366 7001 ext. 8691
www.health.canterbury.ac.nz
healthsciences@canterbury.ac.nz

Date:
(Name and postal address of doctor)

Dear

RE: Cancer and Women's Reproductive Health Study

We are carrying out a study on cancer and women's reproductive health. This is a national study and has received approval from the Southern Health and Disability Ethics Committee and the University of Canterbury Human Ethics Committee. Your help with this research would be greatly appreciated.

We wish to survey your patient, ……., who has been diagnosed with ovarian cancer. We will be sending a questionnaire directly to your patient to complete and return to us.

Particular care is taken to safeguard patient confidentiality.

Please phone us at either of the numbers listed below if contacting your patient will be inappropriate or cause significant distress. If we have not heard from you within 2 weeks of the above date we will send the questionnaire to your patient.

Thank you for your help.

Yours sincerely,

Professor Ann Richardson
MBChB PhD FNZCPHM
Professor of Cancer Epidemiology
Private Bag 4800, Christchurch 8140,
New Zealand
Phone: 364 3786

Dr Jacqueline Chesang
MBChB M.Med
Ph.D Student
Private Bag 4800, Christchurch 8140,
New Zealand
Phone: 021 0256 7310

APPENDIX 3: INVITATION LETTER-CASES

School of Health Sciences

Tel: +64 3 366 7001 ext. 8691

www.health.canterbury.ac.nz

healthsciences@canterbury.ac.nz

Date:

(Name and address of participant)

Dear

RE: Cancer and Women's Reproductive Health Study

We are carrying out a study on cancer and women's reproductive health. This is a national study and has received approval from the Southern Health and Disability Ethics Committee and the University of Canterbury Human Ethics Committee.

We are personally inviting you to take part in this study because you have had recent contact with the health system. We need to include information from women who have not had health issues, and those who have had health problems including cancer. Your help in this study would be greatly appreciated.

With this letter is an information sheet, a consent form, a questionnaire and a prepaid addressed envelope. We would be very grateful if you could read the information sheet and consent form. If you decide to participate please complete the questionnaire and post it back to us together with the signed consent form in the envelope provided.

If you have any questions about this study please feel free to contact us (our contact details are provided on the information sheet).

We sincerely hope you will agree to take part in this study. Thank you for your consideration.

Yours sincerely

Professor Ann Richardson

MBChB PhD FNZCPHM

Professor of Cancer Epidemiology

Dr Jacqueline Chesang

MBChB M.Med

Ph.D Student

APPENDIX 4: INVITATION LETTER-CONTROLS

School of Health Sciences

Tel: +64 3 366 7001 ext. 8691

www.health.canterbury.ac.nz

healthsciences@canterbury.ac.nz

Date:

(Name and address of participant)

Dear

RE: Cancer and Women's Reproductive Health Study

We are carrying out a study on cancer and women's reproductive health. This is a national study and has received approval from the Southern Health and Disability Ethics Committee and the University of Canterbury Human Ethics Committee.

We are personally inviting you to take part in this study. We have learned your name and contact address through the electoral roll. We need to include information from women who have not had health issues, and those who have had health problems including cancer. Your help in this study would be greatly appreciated.

With this letter is an information sheet, a consent form, a questionnaire and a prepaid addressed envelope. We would be very grateful if you could read the information sheet and consent form. If you decide to participate please complete the questionnaire and post it back to us together with the signed consent form in the envelope provided.

If you have any questions about this study please feel free to contact us (our contact details are provided on the information sheet).

We sincerely hope you will agree to take part in this study. Thank you for your consideration.

Yours sincerely

Professor Ann Richardson

MBChB PhD FNZCPHM

Professor of Cancer Epidemiology

Dr Jacqueline Chesang

MBChB M.Med

Ph.D Student

APPENDIX 5: INFORMATION SHEET

School of Health Sciences

Tel: +64 3 366 7001 ext. 8691

www.health.canterbury.ac.nz

healthsciences@canterbury.ac.nz

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

INFORMATION FOR PARTICIPANTS

Title: Cancer and Women's Reproductive Health Study

Principal investigator: Professor Ann Richardson**.**

Additional investigators: Professor John Potter, Mrs. Pat Coope, Dr Mary Jane Sneyd, and Dr Jacqueline Chesang,

Introduction

We are personally inviting you to take part in a nationwide case-control study being carried out at the University of Canterbury School of Health Sciences in partnership with the University of Otago Medical School. The study aims to investigate effects of life habits on women's health including the risk of cancer.

Participation

Your participation is entirely voluntary (your choice). You do not have to take part in this study, and if you choose not to take part this will not affect any future care or treatment provided to you. If you do agree to take part you are free to withdraw from the study at any time, without having to give a reason. You can withdraw any information you have provided, until the information from your questionnaire has been added to the others collected, and the results analysed. Because the information you provide will be anonymous when it is analysed, it cannot be retrieved after that.

When completing the questionnaire, you do not have to answer every question, but we would be very grateful for as much information as you can provide, because all information is valuable. We understand that you may find some of the questions to be of a sensitive nature. The questionnaire is confidential, and you will not be identified as a participant.

About the study

The study will involve over 1,000 New Zealand women. Each woman will complete a questionnaire and post it back to the investigators using a prepaid envelope. It is expected that it will take you about 30 minutes to complete the questionnaire.

Benefits, risks and safety

We expect the results of this study to be beneficial for women's health in New Zealand. There is no risk involved in participating in this study. No blood samples or other specimens will be taken. The study will be done at no cost to the participant.

General

You may have a friend, family or whānau support to help you understand the risks and/or benefits of this study and any other explanation you may require.

If you have any questions or concerns regarding your rights as a participant in this study, you may wish to contact an independent health and disability advocate. This is a free service provided under the Health and Disability Commissioner Act. Free phone: 0800 555 050. Free fax: 0800 2 SUPPORT (0800 2787 7678). Email:advocacy@hdc.org.nz.

You can also address your concerns to:

The Chair, UC Human Ethics Committee, University of Canterbury,

Private Bag 4800, CHRISTCHURCH.

Email: human-ethics@canterbury.ac.nz

Confidentiality

No information that could personally identify you will be used in any reports on this study. The research records will only be accessible to the investigators and the research administrator. The research team will treat the information confidentially.

Results

At the end of this study, which is likely to take 3-4 years, the results are likely to be published in a medical journal. If you would like a copy of the results to be sent to you at the end of the study please indicate this on the consent form.

Statement of approval

This study has received ethical approval from the Southern Ethics Committee, which reviews national and multi-regional studies, ethics reference number: 13/STH/26 and the University of Canterbury Human Ethics Committee.

If you have any questions about this study please feel free to contact the following:

Jacqueline Chesang

University of Canterbury

School of Health Sciences

Private Bag 4800, Christchurch 8140.

Phone: 021 0256 7310

Email: jjc95@uclive.ac.nz

Ann Richardson

University of Canterbury

School of Health Sciences

Private Bag 4800, Christchurch 8140.

Phone: 03 364 3786

Email:ann.richardson@canterbury.ac.nz

APPENDIX 6: CONSENT FORM

School of Health Sciences

Tel: +64 3 366 7001 ext. 8691

www.health.canterbury.ac.nz

healthsciences@canterbury.ac.nz

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

CONSENT FORM

Title: Cancer and Women's Reproductive Health Study

Principal investigator: Professor Ann Richardson

Additional investigators: Professor John Potter, Mrs Pat Coope, Dr. Jacqueline Chesang, and Dr. Mary Jane Sneyd.

- I have read and I understand the information sheet for volunteers taking part in the cancer and women's reproductive health study. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.
- I have had the opportunity to use whānau support or a friend to help me ask questions and understand the study
- I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect my continuing or future health care.
- I understand that my participation in this study is confidential and that no material that could identify me will be used in any reports on this study.
- I have had time to consider whether to take part in the study.
- I know who to contact if I have any questions about the study.
- I note that the project has been reviewed and approved by the Health and Disability Ethics Committee and the University of Canterbury Human Ethics Committee.
- I wish to receive a copy of the results. Yes ☐ No ☐

I _____(full names)

Hereby consent to take part in this study.

Signature_____
Date_____

We would be very grateful if you could please set aside some time to fill in the attached questionnaire.

APPENDIX 7: QUESTIONNAIRE

School of Health Sciences

Tel: +64 3 366 7001 ext. 8691
www.health.canterbury.ac.nz
healthsciences@canterbury.ac.nz

CANCER AND WOMEN'S REPRODUCTIVE HEALTH STUDY

Please follow the following guidelines in filling the questionnaire:

- *Please clearly mark your answers on the appropriate box/boxes.*
- *Put numbers in the appropriate boxes, for example 15th May 1995*

1	5	/	0	5	/	1	9	9	5

- *Print your answers in capital letters in the spaces provided.*
- *Please answer every question as completely as possible.*
- *If you cannot remember the exact date or age, please give an approximate answer.*

SECTION A: GENERAL QUESTIONS ABOUT YOU

1. When were you born? *(Please put day/month/year)*

☐☐/☐☐/☐☐☐☐

2. How old are you? ☐☐ years

3. Where do you usually live?

Suburb or rural locality ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

City, town or district ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Country ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

4. Which country were you born in?

☐ New Zealand

☐ Australia

☐ England

☐ Scotland

☐ China (People's Republic of)

☐ India

☐ South Africa

☐ Samoa

☐ Cook Islands

☐ Other *(Please print the present name of the country*)

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

5. If you live in New Zealand but you were **not** born here, answer this question.

When did you first arrive to live in New Zealand?

Month if known (e.g 03)	Year (e.g 1990)
☐☐	☐☐☐☐

6. Which ethnic group do you belong to?

(Mark the space or spaces which apply to you)

- ☐ New Zealand European
- ☐ Māori
- ☐ Samoan
- ☐ Cook Island Māori
- ☐ Tongan
- ☐ Niuean
- ☐ Chinese
- ☐ Indian
- ☐ Other such as DUTCH, JAPANESE, TOKELAUAN. Please state:

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

SECTION B: QUESTIONS ABOUT YOUR USE OF CONTRACEPTIVES (FAMILY PLANNING METHODS)

To answer this section, use the calendar of life events provided to aid you in recall. Please indicate all the contraceptive methods you have ever used

CALENDAR OF MAJOR LIFE EVENTS

LIFE EVENT	**Year the event occurred**	**How old were you when the event occurred?**	**Type of contraceptive method/s (family planning method/s) you used after the event.**
The first menstrual period in your life			
The first time you had sex			
Birth of 1st child			
Birth of 2nd child			
Birth of 3rd child			
Birth of 4th child			
Birth of 5th child			
Birth of 6th child			
Birth of 7th child			
Birth of 8th child			
Birth of 9th child			
Birth of 10th child			
Menopause (the last menstrual period in your life)			***What contraceptive method were you on around the time of your menopause?***

7. Have you ever used oral contraceptive pills? *(this includes 'the pill' and the mini-pill)*

☐ No *If **No,** please go to question 10*

☐ Yes

If **YES**:

-about how old were you when you first went on the pill? ☐☐ years.

-are you still on the pill? ☐ No, stopped – If so, when? ☐☐ years ago

☐ Yes, still using

-for how many years in total did you take the pill? ☐☐ years

(Add together the years and months when you actually took the pill.

Please write "0" if you used the pill for less than a year in total)

8. Have you ever used oral contraceptive for any other purpose other than as a family planning method? *(e.g. endometriosis, acne etc)*

☐ No *If **No,** please go to question 10*

☐ Yes

If **YES**, please state the reason(s) for using it

……

……..

……..

For how many years in total did you use the pill for the reason(s) stated above?

☐☐ Years

9. For how many years in total have you used the pill (for any other purpose and as a family planning method).

☐☐ Years

(Add together the time you took the pill for other purpose indicated in question 8 above to the time used as a family planning method. Please write "0" if you used the pill for less than a year in total)

10. Have you ever used DMPA? *(depot medroxyprogesterone acetate, a 3 monthly injection, also known as depo-provera)*

☐ No *If **No,** please go to question 11*

☐ Yes

If **YES**:

-about how old were you when you started using DMPA? ☐☐ years.

-are you still on DMPA? ☐ No, stopped – If so, when? ☐☐ years ago

☐ Yes, still using

-for how many years in total did you use DMPA? ☐☐ Years

(Add together the years and months when you actually were on DMPA.

Please write "0" if you used DMPA for less than a year in total)

11. Have you ever used contraceptive implants? *(inserted below the skin on the upper arm)*

☐ No *If **No,** please go to question 12*

☐ Yes

If **YES**:

-about how old were you when you first had a contraceptive implant inserted?

☐☐ years.

-are you still on a contraceptive implant?

☐ No, stopped – If so, when? ☐☐ years ago

☐ Yes, still using

-for how many years in total did you use this method of contraception?

☐☐ Years

(Add together the years and months when you had a contraceptive implant. Please write "0" if you used contraceptive implants for less than a year in total)

-what type of implants have you ever used?

☐ Norplant

☐ Jadelle

☐ Implanon

☐ Zarin

☐ Other (*specify)* ☐☐☐☐☐☐☐☐☐☐☐☐☐☐

☐ I don't know the type of implant I used

12. Have you ever used an intra-uterine contraceptive device? *(IUCDs/coil/loop)*

☐ No *If **No,** please go to question 13*

☐ Yes If **YES**:

-about how old were you when you first had an IUCD inserted? ☐☐ years.

-are you still using an IUCD? ☐ No, stopped – If so, when? ☐☐years ago

☐ Yes, still using

-for how many years in total did you use an IUCD? ☐☐ years

(Add together the years and months when you used an IUCD.

Please write "0" if you used IUCDs for less than a year in total)

- What type of IUCD(s) have you used?

☐ Copper T/copper 7

☐ Multiload

☐ Mirena

☐ Other (*specify)* ☐☐☐☐☐☐☐☐☐☐☐☐☐☐

☐ I don't know the type of IUCD I used.

13. How many sexual partners have you had in your life?

☐☐ Partners

14. Have you ever had a sexual partner who has had a vasectomy?

☐ No *If **No,** please go to question 15*

☐ Yes

If **YES**:

-for how many years in total have you relied on vasectomy for family planning

☐☐ Years

(Add together the years and months you relied on vasectomy for family planning. Please write "0" if the total time is less than a year.)

15. Have you ever used male or female condoms as a family planning method?

☐ No *If **No,** please go to question 16*

☐ Yes

If **YES**:

-for how many years in total did you use this method of contraception?

☐☐ Years

16. Have you ever used any other type of contraceptive not included in the above questions?

☐ No *If **No,** please go to question 17*

☐ Yes – **If yes**, please fill in the following details:

Type of contraceptive	**Your age when you started using it**	**Your age when you stopped using it** *(put "0" if you are still using it)*	**Years in total that you have been on this contraceptive** *(put ''0" if less than one year)*

SECTION C: QUESTIONS ABOUT YOU AND YOUR FAMILY

17. Have your periods **NOW** stopped?

*Cross "**No**" – if you are still having regular periods now, even if they are because you are taking HRT.*

*Cross **"irregular**" – if your periods have been irregular and you think it might be because of the menopause*

*Cross "**Yes"** – if you are not having periods now, either because of your menopause, after hysterectomy (removal of the womb) or after stopping HRT.*

☐ **No**

☐ **Irregular**

☐ **Yes**- If **Yes**, how old were you when they **stopped**? ☐☐ Years

18. Other than the times you were on hormonal contraceptives, would you describe your periods as regular or irregular?

☐ Regular *(number of days between one period and the next is almost fixed, variations of two to three days allowed)*

☐ Irregular *(number of days between one period and the next varies*)

19. What is/was the average number of days between your periods? (*this is from the first day after your period to the last day of your next period)*

☐☐ Days

20. How many children have you had? *(please include stillbirths*) ☐☐ Children

21. When was each child born, and for how many months did you breastfeed each child, if at all?

BIRTH ORDER	**DATE OF BIRTH** (if you had twins or triplets please repeat the same date for each child)			**BREASTFEEDING** *(months that you breastfed each child; put "0" if you did not breastfeed that child and "1" if you breastfed for one month or less)*
	Day	Month	Year	
1st child	☐☐/	☐☐/	☐☐☐☐	☐☐ months
2nd child	☐☐/	☐☐/	☐☐☐☐	☐☐ months
3rd child	☐☐/	☐☐/	☐☐☐☐	☐☐ months
4th child	☐☐/	☐☐/	☐☐☐☐	☐☐ months
5th child	☐☐/	☐☐/	☐☐☐☐	☐☐ months
6th child	☐☐/	☐☐/	☐☐☐☐	☐☐ months
7th child	☐☐/	☐☐/	☐☐☐☐	☐☐ months
8th child	☐☐/	☐☐/	☐☐☐☐	☐☐ months
9th child	☐☐/	☐☐/	☐☐☐☐	☐☐ months
10th child	☐☐/	☐☐/	☐☐☐☐	☐☐ months

22. Have you had any spontaneous miscarriages or induced abortions?

☐ No

☐ Yes If **Yes**, how many? ☐☐ Abortions/ Miscarriages.

23. Has any member of your family been diagnosed with cancer?

☐ No

☐ Yes

*If **YES**, please fill in the following details*

How are you related *(e.g this person is my uncle, aunt, niece, cousin)*	Type of cancer *(e.g. prostate, colon,stomach)*	How old was she/he when the diagnosis was made? (*give age in years)*

SECTION D: QUESTIONS ABOUT YOUR HEALTH

24. Have you had a hysterectomy (your womb removed)?

☐ No

☐ Yes – **If yes**, how old were you? ☐☐ Years

25. Have you had **ONE** ovary removed?

☐ No

☐ Yes – **If yes**, how old were you? ☐☐ Years

26. Have you had **BOTH** ovaries removed?

☐ No

☐ Yes – **If yes**, how old were you? ☐☐ Years

27. Have you been sterilised *(had your tubes tied/clipped)*?

☐ No *If **No,** please go to question 28*

☐ Yes

If yes:

-how old were you when you were sterilised? ☐☐ Years

Has the sterilisation been reversed?

☐ No

☐ Yes

If yes: - how old were you when it was reversed? ☐☐ Years

28. Have you ever been diagnosed with any type of cancer?

☐ No

☐ Yes

*If **YES**, please fill in the following details*

Type of cancer *(for example, ovary, womb/uterus, bowel, breast, etc)*	Your age when the diagnosis was first made *(give age in years)*

29. Have you been told by your doctor that you have:

	No	Yes	Age first diagnosed
Uterine fibroids?	☐	☐	☐☐ years old
Endometriosis?	☐	☐	☐☐ years old
Benign ovarian cysts?	☐	☐	☐☐ years old
Infertility?	☐	☐	☐☐ years old

30. Have you ever used drugs that promote ovulation to overcome difficulty conceiving a child?

☐ No

☐ Yes

If **YES**, what is/was the total length of time you used it?

☐☐ Years and ☐☐ Months

31. Have you ever used HRT (hormone replacement therapy, also known as post-menopausal hormone)?

☐ No

☐ Yes – **If yes**, how many years in total? ☐☐ total years of use

(Please put "0" if you used/have used HRT for less than one year in total)

SECTION E: MORE QUESTIONS ABOUT YOURSELF

32. What is your highest secondary school qualification?

☐ None

☐ NZ School Certificate in one or more subjects *or*

National Certificate Level 1 *or*

NCEA level 1

☐ NZ Sixth Form Certificate in one or more subjects *or*

National Certificate level 2 *or*

NZ UE before 1986 in one or more subjects *or*

NCEA level 2

☐ NZ Higher School Certificate *or*

Higher Leaving Certificate

NZ University Bursary/Scholarship *or*

National Certificate level 3 *or*

NCEA level 3 *or*

NZ Scholarship

☐ Other secondary school qualification **gained in NZ**. Print what it is:

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Or ☐ other secondary school qualification **gained overseas**.

33. Apart from secondary school qualifications, do you have another completed qualification?

(*Don't count qualifications that take less than 3 months of full-time study to get*)

☐ No

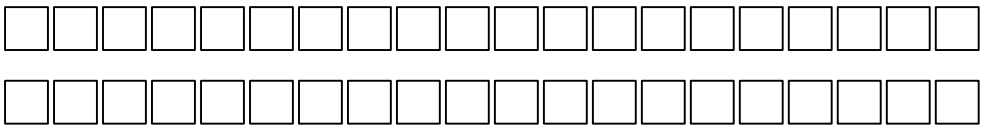
☐ Yes

If **YES**, print your highest qualification and the main subject; for example:

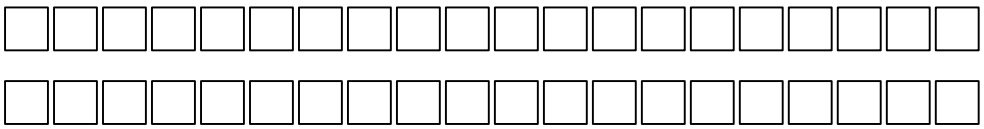
Qualification: TRADE CERTIFICATE

Subject: ELECTRICAL ENGINEERING

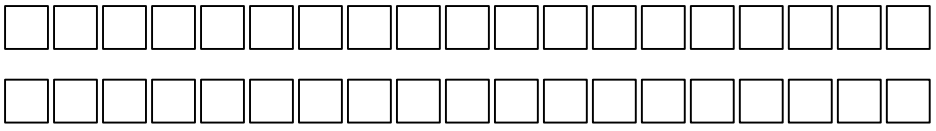
Qualification (and level, if applicable)

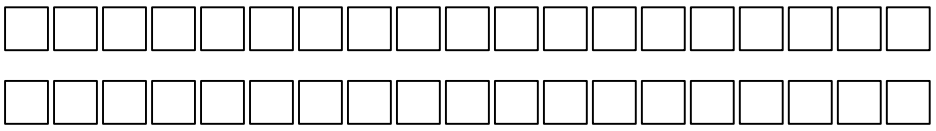


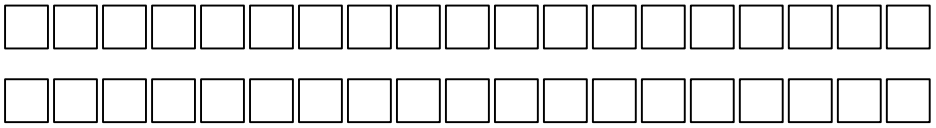
Subject



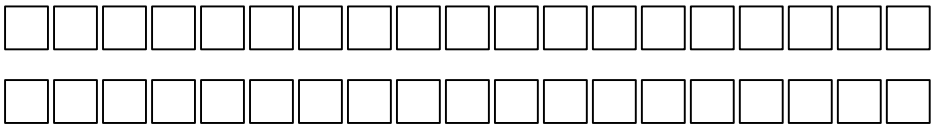
34. What is your main occupation? (for example PRIMARY SCHOOL TEACHER, CLOTHING MACHINIST, MOTEL MANAGER, RECEPTIONIST etc. *Please list if you have more than one main occupation*)

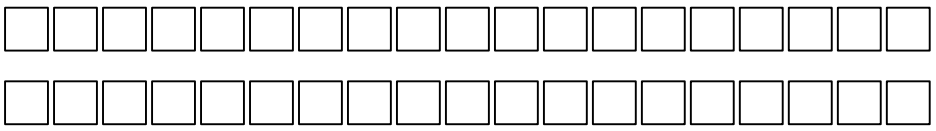
i. 

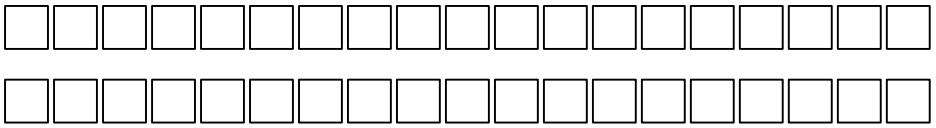
ii. 

iii. 

35. If you are retired, what was your main occupation before retirement (*Please list if you had more than one main occupation*)?

i. 

ii. 

iii. 

36. From all your sources of income, what will the total income be :

- That you yourself got
- Before tax or anything was taken out of it
- In the 12 months ending 31st March this year?

☐ Loss

☐ Zero income

☐ $1-$5,000

☐ $5,001-$10,000

☐ $10,001-$15,000

☐ $15,001-$20,000

☐ $20,001-$25,000

☐ $25,001-$30,000

☐ $30,001-$35,000

☐ $35,001-$40,000

☐ $40,001-$50,000

☐ $50,001-$60,000

☐ $60,001-$70,000

☐ $70,001-$100,000

☐ $100,001-$150,000

☐ $150,001 or more

37. In the last 10 years, about how much wine, beer or spirits did you usually drink a week? (*Please cross one box for each type*)

Wine (glasses per week)	**Lager/Cider/Beer** (half pints per week)	**Spirits** (tots per week)
☐ none	☐ none	☐ none
☐ less than 1	☐ less than 1	☐ less than 1
☐ 1-3	☐ 1-3	☐ 1-3
☐ 4-6	☐ 4-6	☐ 4-6
☐ 7-10	☐ 7-10	☐ 7-10
☐ 11-15	☐ 11-15	☐ 11-15
☐ 16-20	☐ 16-20	☐ 16-20
☐ 21+	☐ 21+	☐ 21+

If you drink **wine** is it

☐ Mostly red ☐ mostly white

☐ About the same amount of red and white

38. Have you ever been a regular smoker of one or more cigarettes a day?

☐ No *If **No,** please go to question 42*

☐ Yes

39. How old were you when you started smoking regularly?

☐☐ Years old

40. Are you a smoker now?

☐ No If **No**,how old were you when you stopped smoking?

☐☐ Years old

☐ Yes

41. About how many cigarettes do you/did you smoke on average each day? *(if you are an ex-smoker, how many did you smoke on average when you smoked?)*

☐☐ Cigarettes per day

42. Have you ever applied/used talcum powder on your groin area?

☐ No

☐ Yes – **if yes**, how many years in total? ☐☐ total years of use

(Please put "0" if you used/have used talcum powder for less than one year in total)

43. How tall are you? *(please give to the nearest centimetre/inch)*

☐☐☐ Centimetres. *Or* ☐Feet and ☐☐Inches

44. About how much do you weigh now?

☐☐☐ Kilograms. *Or* ☐☐☐ Pounds

45. What did you weigh at 18 years of age?

☐☐☐ Kilograms. *Or* ☐☐☐Pounds

46. What has been your usual weight after 18 years of age excluding the times when you were pregnant?

☐☐☐ Kilograms. *Or* ☐☐☐Pounds

47. On which date did you fill in this questionnaire?

☐☐/☐☐/☐☐☐☐*(Fill as day/month/year)*

48. We may wish to contact you for further information about this study. If you are agreeable to this, please indicate your telephone contact below.

Telephone number: Area code (_ _ _ _)

Phone number (_ _ _ _ _ _ _ _ _ _ _)

Mobile phone number: _____

What is the best time to call you? _____

49. If you would like to receive a summary of the results of this study, please provide an address or other way for us to send it to you at the end of the study.

_____

_____

_____

Thank you for taking time to fill in this questionnaire. Please post it back to us together with the consent form in the envelope provided.

APPENDIX 8: UC HUMAN ETHICS COMMITTEE APPROVAL LETTER

HUMAN ETHICS COMMITTEE

Secretary, Lynda Griffioen
Email: human-ethics@canterbury.ac.nz

Ref: HEC 2013/08

11 March 2013

Jacqueline Chesang
Health Sciences Centre
UNIVERSITY OF CANTERBURY

Dear Jacqueline

The Human Ethics Committee advises that your research proposal "Ovarian cancer and contraception" has been considered and approved.

Please note that this approval is subject to the incorporation of the amendments you have provided in your email of 7 March 2013.

Best wishes for your project.

Yours sincerely

Lindsey MacDonald
Chair
University of Canterbury Human Ethics Committee

APPENDIX 9: UC HUMAN ETHICS COMMITTEE APPROVAL LETTER

HUMAN ETHICS COMMITTEE

Secretary, Lynda Griffioen
Email: human-ethics@canterbury.ac.nz

Ref: HEC 2013/08

18 December 2013

Jacqueline Chesang
School of Health Sciences
UNIVERSITY OF CANTERBURY

Dear Jacqueline

Thank you for your request for an amendment to your research proposal "Ovarian cancer and contraception" as outlined in your email dated 16 December 2013.

I am pleased to advise that this request has been considered and approved by the Human Ethics Committee.

Yours sincerely

Lindsey MacDonald
Chair, Human Ethics Committee

APPENDIX 10: HDEC APPROVAL LETTER

Health and Disability Ethics Committees

Health and Disability Ethics Committees
1 the Terrace
PO Box 5013
Wellington
6011
0800 4 ETHICS
hdecs@moh.govt.nz

12 April 2013

Professor Ann Richardson
School of Health Sciences
University of Canterbury
Private Bag 4800
Christchurch 8140
Christchurch
8140

Dear Professor Richardson

Re:	**Ethics ref:**	**13/STH/26**
	Study title:	Association of intimate partner vasectomy, use of long-acting progestogen-based contraceptives and intrauterine contraceptive devices with risk of ovarian cancer

I am pleased to advise that this application has been *approved* by the Southern Health and Disability Ethics Committee. This decision was made through the HDEC-Expedited Review pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Southern Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at *a given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

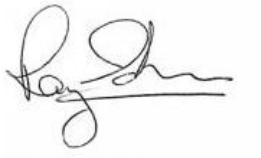
Participant access to ACC

The Southern Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or

distributor of the medicine or item being trialled. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,



Raewyn Idoine
Chairperson
Southern Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted

Document	*Version*	*Date*
CV for CI: Professor Ann Richardson is the Study's Co-ordinating Investigator and primary contact person.	1	28 January 2013
CVs for other Investigators	1	28 January 2013
CVs for other Investigators	1	28 January 2013
CVs for other Investigators	1	28 January 2013
Evidence of scientific review: The study proposal was reviewed by Genesis Oncology Trust Assessment Committee in the 2012 grant round.	1	28 January 2013
This is the consultation that was sent to the University of Canterbury Maori Research Advisory Group.	1	28 January 2013
Attached is the response we received from the University of Canterbury Maori Research Advisory Group.	1	28 January 2013
Attached is the correspondence we have had with the New Zealand Cancer Registry.	1	28 January 2013
Attached is the correspondences we have had with the Electoral Enrolment Centre.	1	28 January 2013
This confidentiality agreement is to be signed by the Research Administrator.	1	28 January 2013
This form will be completed with information obtained from the Cancer Registry.	1	28 January 2013
This letter will be sent to the doctors of women with ovarian cancer (cases) requesting approval to approach their patients about the study.	Version 1	01 March 2013
This letter will be sent to women without ovarian cancer (controls) inviting them to participate in the study.	Version 1	01 March 2013
This letter will be sent to women with ovarian cancer (cases) inviting them to participate in the study.	Version 1	01 March 2013
PIS/CF: This consent form will be used by all the participants (both cases and controls)	1	01 March 2013
Survey/questionnaire: This a self-administered questionnaire that will be completed by all study participants (both cases and controls)	1	01 March 2013
Protocol	1	01 March 2013
PIS/CF: A copy of this information sheet will be sent to all participants (both cases and controls).	1	01 March 2013
Application	1	19 March 2013
PIS/CF: Participant Information Sheet	1	01 March 2013
Response to Request for Further Information	1	04 April 2013

Appendix B
Statement of compliance and list of members

Statement of compliance

The Southern Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008713) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	*Category*	*Appointed*	*Term Expires*
Ms Raewyn Idoine	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Mr Doug Bailey	Lay (the law)	01/07/2012	01/07/2015
Mrs Angelika Frank-Alexander	Lay (consumer/community perspectives)	01/07/2012	01/07/2014
Dr Sarah Gunningham	Non-lay (intervention studies)	01/07/2012	01/07/2015
Ms Gwen Neave	Lay (consumer/community perspectives)	01/07/2012	01/07/2014
Dr Nicola Swain	Non-lay (observational studies)	01/07/2012	01/07/2014
Dr Martin Than	Non-lay (intervention studies)	01/07/2012	01/07/2014
Dr Mathew Zacharias	Non-lay (health/disability service provision)	01/07/2012	01/07/2015

http://www.ethics.health.govt.nz

APPENDIX 11: HDEC APPROVAL LETTER

Health and Disability Ethics Committees

Health and Disability Ethics Committees
C/- MEDSAFE, Level 6, Deloitte House
10 Brandon Street
PO Box 5013
Wellington

0800 4 ETHICS
hdecs@moh.govt.nz

21 January 2014

Professor Ann Richardson
School of Health Sciences
University of Canterbury
Private Bag 4800
Christchurch 8140
Christchurch 8140

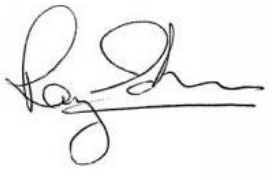
Dear Professor Richardson

Re:	**Ethics ref:**	**13/STH/26/AM01**
	Study title:	Association of intimate partner vasectomy, use of long-acting progestogen-based contraceptives and intrauterine contraceptive devices with risk of ovarian cancer

I am pleased to advise that this amendment has been ***approved*** by the Southern Health and Disability Ethics Committee. This decision was made through the HDEC Expedited Review pathway.

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,



Ms Raewyn Idoine
Chairperson
Southern Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted

Document	Version	Date
Survey/questionnaire: Questionnaire that will be sent to all the participants (cases and controls)	2	01 December 2013
Letter that will be sent to doctors of women with ovarian cancer.	2	01 December 2013
Invitation letter to women with ovarian cancer (cases).	2	01 December 2013
Invitation letter to controls (women without ovarian cancer).	2	01 December 2013
Information sheet to be sent to all the participants (both cases and controls).	2	01 December 2013
Consent form to be sent to all participants (both cases and controls).	2	01 December 2013
Post Approval Form		16 December 2013

Appendix B
Statement of compliance and list of members

Statement of compliance

The Southern Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
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- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
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List of members

Name	*Category*	*Appointed*	*Term Expires*
Ms Raewyn Idoine	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Mrs Angelika Frank-Alexander	Lay (consumer/community perspectives)	01/07/2012	01/07/2014
Dr Sarah Gunningham	Non-lay (intervention studies)	01/07/2012	01/07/2015
Ms Gwen Neave	Lay (consumer/community perspectives)	01/07/2012	01/07/2014
Dr Nicola Swain	Non-lay (observational studies)	01/07/2012	01/07/2014
Dr MARTIN THAN	Non-lay (intervention studies)	01/07/2012	01/07/2014
Dr Devonie Waaka	Non-lay (intervention studies)	01/07/2013	01/07/2016
Dr Mathew Zacharias	Non-lay (health/disability service provision)	01/07/2012	01/07/2015

http://www.ethics.health.govt.nz

APPENDIX 12: COMPARISON OF SOCIO-DEMOGRAPHIC CHARACTERISTICS AND RISK FACTORS FOR OVARIAN CANCER OF CASES VERSUS CONTROLS

Table 1: Comparisons of socio-demographic characteristics between cases and controls (χ^2 test)

	Cases		Controls		Chi-Square test		P-Value
	No.	*(%)**	*No.*	*(%)**	χ^2	*df*	
Socio-demographic characteristics							
Age							
35-39	5	(3)	43	(6)			
40-44	12	(8)	61	(8)			
45-49	18	(12)	91	(12)			
50-54	35	(23)	94	(13)	12.198	235	0.058
55-59	29	(19)	156	(21)			
60-64	27	(18)	152	(20)			
65-69	26	(17)	149	(20)			
Country of Birth							
Not born in NZ	33	(22)	159	(21)	0.100	1	0.920
Born in NZ	119	(78)	586	(79)			
Ethnicity							
Māori	13	(9)	59	(8)			
Pacific	7	(5)	17	(2)	2.747	2	0.253
Other (non-Māori non-Pacific)	132	(87)	670	(90)			
Education							
No qualification	16	(11)	95	(13)			
Overseas Secondary school qualification	3	(2)	28	(4)			
Level 1 or 2 Certificate	37	(25)	159	(21)	8.463	6	0.206
Level 3 or 4 Certificate	35	(24)	119	(16)			
Level 5 or 6 Diploma	21	(14)	113	(15)			
Bachelors Degree & Level 7 qualification	28	(19)	162	(22)			
Postgraduate	8	(5)	64	(9)			
Income							
≤$20,000 (Loss-$20,000)	34	(25)	186	(26)			
$20,001-$40,000	39	(29)	179	(25)	4.270	4	0.371
$40,001-$60,000	33	(24)	142	(20)			
$60,001-$100,000	25	(18)	143	(20)			
>$100,000 ($100,001 or More)	5	(4)	54	(8)			

*Percentages are of total stated

Table 2: Comparison of reproductive factors and ever-use of contraceptives between cases and controls (χ^2 test)

	Cases		Controls		Chi-Square test		P-Value
	No.	*(%)**	*No.*	*(%)**	χ^2	*df*	
Reproductive factors							
Period Ceased							
No	46	(30)	229	(31)	0.016	1	0.900
Yes	106	(70)	515	(69)			
Menopause Type							
Natural	82	(78)	421	(79)	0.075	1	0.784
Surgical	23	(22)	110	(21)			
Menstrual Regularity							
Regular	124	(82)	607	(82)	0.001	1	0.979
Irregular	27	(18)	133	(14)			
Abortions							
No	107	(71)	471	(63)	3.200	1	0.074
Yes	44	(29)	274	(37)			
Contraceptives							
Oral Contraceptives							
Ever-Use							
No	36	(24)	77	(10)	20.497	1	<0.001
Yes	116	(76)	669	(90)			
OC Use Ever-Other							
No	100	(86)	550	(82)	0.984	1	0.321
Yes	16	(14)	117	(18)			
DMPA							
Ever-Use							
Yes	13	(9)	88	(12)	1.342	1	0.247
No	139	(91)	657	(88)			
IUDs							
Ever-Use							
No	112	(74)	554	(74)	0.030	1	0.862
Yes	40	(26)	191	(26)			
Vasectomy							
Vasectomy Ever-Use							
No	98	(64)	412	(55)	4.200	1	0.039
Yes	54	(36)	332	(45)			
Condoms							
Condoms Ever-Use							
No	82	(54)	365	(49)	1.239	1	0.266
Yes	70	(46)	380	(51)			
Tubal Ligation							
Ever had TL							
No	121	(82)	600	(80)	0.140	1	0.709
Yes	27	(18)	146	(20)			

*Percentages are of total stated

Table 3: Comparisons of other risk factors for ovarian cancer between cases and controls (χ^2 test)

	Cases		Controls		Chi-Square test		P-Value
	No.	*(%)**	*No.*	*(%)**	χ^2	*df*	
Gynaecological operations							
Unilateral Oophorectomy							
No	147	(97)	711	(95)	0.583	1	0.445
Yes	5	(3)	35	(5)			
Hysterectomy							
No	134	(88)	630	(84)	1.367	1	0.242
Yes	18	(12)	116	(16)			
Gynaecological conditions and cancer							
Fibroids							
No	126	(84)	632	(86)	0.267	1	0.605
Yes	24	(16)	106	(14)			
Endometriosis							
No	126	(84)	687	(94)	16.132	1	<0.001
Yes	24	(16)	46	(6)			
Benign Ovarian Cysts							
No	132	(89)	671	(91)	1.087	1	0.297
Yes	17	(11)	64	(9)			
Infertility							
No	135	(91)	704	(96)	9.697	1	0.002
Yes	14	(9)	26	(4)			
Familial predisposition							
No	95	(63)	519	(70)	2.938	1	0.086
Yes	55	(37)	218	(30)			
Any Cancer History							
No	26	(17)	212	(29)	8.224	1	0.004
Yes	125	(83)	531	(71)			
Use of fertility drugs							
No	139	(92)	706	(95)	1.720	1	0.190
Yes	12	(8)	39	(5)			
PMH Use							
No	117	(77)	627	(84)	4.127	1	0.042
Yes	34	(23)	117	(16)			
Lifestyle factors							
Talcum Use							
No	97	(65)	546	(74)	4.672	1	0.031
Yes	52	(35)	194	(26)			
Alcohol Use							
No	35	(23)	126	(17)	3.317	1	0.069
Yes	116	(77)	618	(83)			
Smoking							
Never	91	(60)	443	(59)	0.041	1	0.840
Ever	60	(40)	303	(41)			
Smoking Status							
Never Smoker	91	(60)	443	(59)			
Ex-Smoker (Past Smoker)	48	(32)	236	(32)	0.170	2	0.919
Current Smoker	12	(8)	67	(9)			

*Percentages are of total stated

Table 4: Comparison of risk factors for ovarian cancer between cases and controls (t-test)

	Cases		Controls		t-test		P-Value
	Mean	***(SD)****	***Mean***	***(SD)****	***t***	***df***	
Age	56.0	(8.0)	56.0	(9.0)	-0.414	235	0.679
Reproductive characteristics							
Age at Menarche	13.0	(1.5)	12.9	(1.5)	0.295	880	0.773
Age at sexual debut	19.2	(3.9)	18.7	(3.3)	1.383	187	0.168
Number of partners	5.7	(9.9)	5.5	(7.5)	0.370	848	0.711
Age at menopause	48.4	(5.7)	48.3	(6.8)	0.213	278	0.831
Cycle length	27.8	(5.9)	27.5	(5.3)	-0.506	790	0.613
Parity	1.9	(1.4)	2.3	(1.3)	-3.221	894	0.001
Age at first delivery	25.6	(5.3)	26.1	(5.6)	-1.066	771	0.287
Age at last delivery	29.6	(5.4)	31.2	(5.6)	-3.015	771	0.003
Breastfeeding – Months (Parous)	16.5	(16.2)	21.8	(22.6)	-3.053	211	0.015
Years of Ovulatory Cycles	28.8	(9.4)	23.0	(11.0)	5.389	151	<0.001
Gynaecological operations							
Hysterectomy - Age	43.6	(6.4)	41.8	(8.1)	0.898	132	0.371
Unilateral oophorectomy - Age	39.2	(14.9)	38.7	(10.5)	0.098	38	0.923
Fertility drugs use - Months	4.1	(4.3)	2.6	(15.8)	-2.946	47	0.005
PMH Use -Years	3.7	(4.7)	6.0	(6.8)	-1.828	149	0.070
Lifestyle factors							
Years Smoked	24.7	(15.4)	21.7	(14.1)	1.476	356	0.141
Pack Years	16.0	(16.0)	13.8	(13.1)	1.006	75.8	0.251
Years Last smoked	15.7	(13.9)	22.0	(12.3)	-3.165	275	0.002
Talcum Use Years	14.6	(17.2)	16.6	(19.8)	-0.659	225	0.511
Anthropometric measures							
Height (cm)	164.3	(8.2)	164.2	(7.3)	0.127	886	0.899
Current Weight (Kgs)	69.9	(16.6)	73.3	(16.5)	-2.292	883	0.022
Current BMI (Kg/M^2)	25.9	(5.8)	27.2	(5.9)	-2.460	876	0.014
Teen Weight (Kgs)	59.9	(10.9)	59.9	(12.4)	0.031	819	0.975
Teen BMI	22.2	(3.8)	22.2	(4.2)	0.076	813	0.939
Usual Weight	68.5	(15.7)	67.0	(13.6)	1.250	849	0.211
Usual BMI	25.4	(5.6)	24.8	(4.7)	1.195	189	0.234
Contraceptives							
Oral Contraceptives							
Age 1^{st} Use	19.9	(4.6)	19.4	(3.5)	1.199	138	0.233
Years Since Last Use	26.4	(10.0)	24.0	(11.4)	2.313	167	0.022
OCs Use as Contraceptive-Years	8.0	(8.0)	11.0	(8.4)	-3.466	768	0.001
OCs 'Other' Years of Use	4.6	(7.0)	5.2	(6.9)	-0.334	128	0.739
All Use OCs years	7.8	(7.5)	11.0	(8.3)	-3.781	765	<0.001
DMPA							
Age 1^{st} Use	24.1	(7.8)	24.4	(6.1)	-0.181	97	0.857
Years Since Last Use	25.9	(9.3)	25.3	(12.4)	0.184	90	0.854
Years of Use	3.1	(4.1)	4.5	(6.3)	-0.771	95	0.442
IUDs							
Age 1^{st} Use	29.3	(8.3)	29.0	(7.7)	0.184	226	0.854
Years Since Last Use	15.7	(12.9)	24.9	(12.0)	-4.099	193	<0.001
Years of Use	8.4	(9.1)	5.6	(5.8)	1.806	42	0.078
Vasectomy							
Years Reliant on Vasectomy	12.9	(11.3)	15.3	(10.3)	-1.480	376	0.140
Condoms							
Condoms-Years of Use	7.8	(10.2)	6.1	(7.5)	1.282	78	0.204
Tubal Ligation							
Age TL	33.2	(4.8)	33.0	(5.9)	0.197	171	0.844
Years Since TL	25.8	(9.7)	28.0	(9.5)	-1.126	171	0.262

*SD = standard deviation